THE MECHANISM OF GALLBLADDER INFECTIONS IN LABORATORY ANIMALS

EXPERIMENTAL TYPHOID-PARATYPHOID CARRIERS. V

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Experimental gallbladder infections have been produced in the rabbit, guinea-pig, etc., by numerous investigators, following intravenous injections of typhoid, paratyphoid, dysentery and coli bacilli, streptococci, staphylococci and even nonpathogenic organisms. A perusal of the numerous publications indicates that these infections of the biliary system are, however, not developed with any degree of certainty. In spite of the excellent experimental studies of Doerr,[1] Blumenthal,[2] Else,[3] Nichols [4] and others, our knowledge is still incomplete concerning the exact mechanism which provokes in the rabbit a localization of the inoculated typhoid bacilli. Moreover, it has been customary to look on the gallbladder as the only great breeding place of typhoid bacilli, whenever they have been found in this viscus a few weeks after the inoculation of the infective dose. No doubt there is a considerable accumulation of data pointing in this direction, but there are observations on rabbits and guinea-pigs, namely, those of Hailer and his associates,[5] and Emmerich and Wagner,[6] which do not conform to this conception of the carrier state. These workers have demonstrated that in about 10% of instances the cystic bile may be found sterile, although the wall contains typhoid bacilli. The biliary passages may likewise be sterile and yet the liver (in 80% of the instances), the bone-marrow (in 25 to 50% of the instances) and the spleen (in 38% of the instances) may harbor the bacilli for more than 30 days. Moreover, the majority of experimental pathologists who study the gallbladder carrier state in rabbits, overlook the early observation of

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[1] Centralbl. f. Bakteriol., I., 1905, 39, p. 624.
[2] Ibid., 1910, 55, p. 341.
[3] Surg., Gynec. & Obst., 1910, 11, p. 470.
[4] Jour. Exper. Med., 1914, 20, p. 573; 1916, 24, p. 497; 1917, 68, p. 958.
[5] Deutsch. med. Wchnschr., 1912, 38, p. 2267; Arb. a. d. k. Gsndhtsamte, 1914, 47, pp. 303, 451, and 470. Hailer, E., and Wolf, G.: Arb. a. d. k. Gsndhtsamte, 1914-15, 48, p. 80.
[6] Centralbl. f. allg. Path. u. path. Anat., 1916, 27, p. 433.

Blachstein;[7] namely, in chronic or subacute infections of the gallbladder contents the lesions from which the infection occurs exist in the liver in the form of necrotic or inflammatory foci. Keeping these facts in mind, it seems advisable to regard the biliary tract as an excretory channel and the cholecystitis more as a sequel than as the source of the continual and repeated infection of the bile. A similar but novel view has recently been advanced by Webb-Johnson[8] to explain the human intestinal carrier state. This writer assumes the spleen to be the primary focus responsible for the elimination of typhoid bacilli in the bile. Opportunity will be afforded to discuss these conceptions in the course of the analysis of our experimental data.

Following an intravenous injection of typhoid bacilli, such organisms can be demonstrated quite regularly in the gallbladder, but the duration of their sojourn is exceedingly variable and in many instances limited to a short period. A study of the pathogenesis of the experimental carrier state in laboratory animals must therefore consider (a) the mechanism by which the bacilli reach the gallbladder and (b) the concomitant factors in the form of anatomic or physiologic changes, which either lead to a prolonged persistence or to a rapid disappearance of the invading typhoid bacilli. Among these changes may be mentioned: the pathologic processes in the gallbladder wall (cholecystitis and cholangitis); peculiarities in the physiology of the bile with reference to its secretion, composition and reaction; and abnormalities in the biliary system leading to the formation of gallstones. Consideration must also be given to the question of the individual immunity of the infected animal. It is the purpose of this paper to investigate these contributory factors in connection with their bearing on the mechanism of gallbladder infections.

GENERAL CONSIDERATIONS

It is generally stated that theoretically bacteria may reach the gallbladder in the following manner: (a) direct infection (puncture); (b) ascending infection from the intestines via ductus choledochus; (c) descending infection (with the bile secreted from the liver); (d) arterial embolus of the wall vessels; (e) through the lymphatic and venous blood vessels. Each of these possibilities has been investigated experimentally and has been shown to occur in typhoid cholecystitis in

[7] Bull. Johns Hopkins Hosp., 1891, 2, pp. 96 and 121.

[8] The Lancet, 1917, 2, p. 813; Surgical Aspects of Typhoid and Paratyphoid Fevers, London, 1919, p. 167.

the rabbit. Some of the observations have even *sine conditione* been applied to explain human cholecystitis, and the intestinal carrier state. A brief consideration is therefore justifiable to determine, if possible, which of these routes is the one usually followed in the experimental reproduction of gallbladder infection in animals and to what extent the findings are analogous to those present in the human carrier state.

Infection through trauma (puncture) occurs in rare instances only as a result of surgical procedures or manipulations. The direct inoculation of various bacteria into the gallbladder of rabbits and guinea-pigs has been practiced experimentally by Gilbert, and Dominici [9] and Fournier,[10] Talma, Richardson,[11] Italia,[12] Ehret and Stolz,[13] Forster,[14] Violle,[15] Marxer,[16] Uhlenhuth and Messerschmidt,[17] Klinkert,[18] Hailer and Ungermann,[19] Lange and Roos,[20] Schöbl,[21] Emmerich and Wagner,[22] and Venema.[23] The published reports indicate that this method produces with greater regularity a localization of the bacteria in the gallbladder than the intravenous method of infection. Constant results with the typhoid bacillus can be expected in rabbits, according to Hailer and Ungermann, only until the 30th day after the operation. In a few animals the bacilli disappear at the end of this period, while in others a chronic inflammation favors their persistence for from 217 (Hailer and Rimpau) to 341 days (Emmerich and Wagner). The results which are obtained with the typhoid bacillus in guinea-pigs are less satisfactory (Marmoreck.[24] Wagner and Emmerich,[25] Thompson and Meyer [26]). The gallbladder infections

[9] Bull. d. l. Soc. de biol., 1893, 5, p. 1033.

[10] Compt. rend. Soc. de biol., 1897, 49, pp. 692 and 636.

[11] Boston Soc. Med. Sc., 1898-99, 3, p. 79.

[12] Policlin. Roma, 1901, 8.—C., p. 153.

[13] Mitt. a. d. Grenzgeb. d. Med. u. Chir., 1900, 6, p. 350; 1900, 7, p. 372; and 1901, 8, p. 153.

[14] Forster, in Uhlenhuth, P., and Messerschmidt, T.: Deutsch. med. Wchnschr., 1912, 51, p. 2367.

[15] Ann. de l'Inst. Pasteur, 1908, 22, p. 341.

[16] Ztschr. f. Chemotherap., 1914, 2, p. 23.

[17] Deutsch. med. Wchnschr., 1912, 51, p. 2367.

[18] Berl. klin. Wchnschr., 1911, 48, p. 335.

[19] Deutsch. med. Wchnschr., 1912, 38, p. 2267; Arb. a. d. k. Gsndhsamte, 1914, 47, pp. 303, 451 and 470.

[20] Ibid., 1915, 50, p. 57.

[21] Jour. Infect. Dis., 1916, 18, p. 307; 19, p. 145; Philippine Jour. Trop. Med., 1916, 11, p. 153; 1917, 12, p. 23.

[22] Med. Klin., 1916, 12, p. 74; Zschr. f. Immunitatsforsch. u. exper. Therapie, 1916, 24, p. 557; Centralbl. f. allg. Path. u. path. Anat., 1916, 27, p. 433.

[23] Berl. klin. Wchnschr., 1917, 54, p. 815.

[24] Med. Klin., 1916, 12, p. 275.

[25] Centralbl. f. Bakteriol, 1916, 79, p. 1.

[26] To be published.

provoked in rabbits by intracystic infections of cholera vibrios or dysentery bacilli (Schöbl,[21] Nichols,[27] Meyer and Stickel [28]) are characterized by a benign process, which shows a tendency to rapid recovery. The organisms disappear from the injured viscus at the end of from the 15th to the 30th day. All authors, however, agree that direct inoculation of the gallbladder is the route par excellence for the production of experimental gallbladder infections.

It is obvious that the foregoing method does not explain the route by which the typhoid bacillus reaches the gallbladder in the course of the disease. The early finding of typhoid bacilli in the gallbladder of fatal typhoid cases by Gilbert and Girode, Chiari,[30] Pratt, and others, in view of the conception (prevailing at the time of their observations) that typhoid was a purely intestinal disease, was explained by the assumption that the bacillus reached the gallbladder by an ascending route from the intestines through the ductus choledochus. Unquestionably the ingenious experiments of Homén,[31] Ehret and Stolz, Netter, Naunyn, Doerr, Blumenthal and others, which demonstrated the occurrence of bacteria in the bile subsequent to the ligation of the common duct, contributed greatly to the abandonment of this theory as it pertains to the typhoid bacillus. We have been unable, in a number of experiments, to produce gallbladder infections by the introduction of enormous doses of typhoid bacilli into the duodenum close to the papilla of Vater. These results are analogous to those of Hailer and Ungermann. It is needless to state that these findings apply only to the typhoid bacillus, because in the light of numerous clinical and pathologic observations it seems reasonable to suspect an occasional invasion of bacteria or protozoa by the ascending route. Such a mode of infection is particularly favored by intestinal or biliary stasis. The invasion probably never occurs through the common duct against the bile current except in infections with the entameba, but follows the lymphatics of the duct, as has been definitely demonstrated in ascending renal infections; B. coli, B. dysenteriae and Cholera vibrio infections may be mentioned as possible examples of this type of gallbladder infection. In fact, Schöbl [32] has demonstrated experimentally in some guinea-pigs

[27] Jour. Exper. Med., 1916, 24, p. 497.

[28] To be published.

[30] Verhandl. d. Deutsch. path. Gesellschaft, 1907; Ergänz. heft. z. Centralbl. f. allg. Path. u. path. Anat., 1908, 18, p. 143.

[31] Ibid., 1894, 5, p. 825.

[32] J. Infect. Dis., 1916, 18, p. 307.

the presence of cholera vibrios in the bile 7 to 14 days after the feeding of the bacteria.

Descending or so-called hemato-hepatogeneous infection of the bile has been repeatedly verified experimentally and is now generally accepted. Birch-Hirchfeld[33] was the first to state that micro-organisms reach the gallbladder through the circulation either by way of the entero-hepatic blood stream (vena portae) or the arteria hepatica. It is a proved fact that various micro-organisms inoculated intravenously or into a radical of the portal vein of rabbits, dogs, guinea-pigs, and other animals appear in from 2 to 50 minutes in the bile collected from temporary common duct or permanent gallbladder fistulas or from the cystis bile at necropsy. The early studies of v. Fütterer [34] in 1888-1899 on fistular animals have been verified by Biedl and Kraus [35] and by Nichols.[27] Pernice and Scagliosi,[36] Sherrington,[37] Pawlowsky,[38] Métin,[39] Heck [40] and others have inoculated animals subcutaneously, intraperitoneally, or intravenously with various organisms and have tested after varying time intervals the bile or urine for the presence of the inoculated bacteria. The results have been irregular. For example, Sherrington reports that after the injection of anthrax bacilli, staphylococci and pyocyaneus only 18 biles of a series of 49 proved to be infected, while Heck and Métin record negative results. Similarly disappointing have been the experiments of Carmichael [41] and Else,[3] who introduced the bacteria by way of the portal vein. On the other hand, it is stated by Breton, Bruyant and Mezie,[42] that even the introduction of B. prodigiosus by gavage produces in a guinea-pig with a ligated common duct an invasion of the gallbladder bile 3 to 4 hours after the introduction of the bacteria. In our feeding experiments with typhoid bacilli, we have cultivated the bacilli occasionally from the liver, but never from the bile. Even in the course of a successful series of paratyphoid feeding infections, we have not succeeded in demonstrating the bacteria in the bile, although as a rule they have

[33] Lehrbuch d. path. Anat., Ed. 4, 1895, 2, p. 694.
[34] Berl. klin. Wchnschr., 1899, 36, p. 58; Medicine, 1895, 1, p. 279.
[35] Ztschr. f. Hyg. u. Infektionskrankh., 1897, 26, p. 353; and Zentralbl. f. inn. Med., 1896, 17, p. 737.
[36] Deutsch. med. Wchnschr., 1892, 18, p. 761.
[37] Jour. Path. and Bacteriol., 1892-93, 1, p. 259.
[38] Ztschr. f. Hyg. u. Infektionskrankh., 1900, 33, p. 261.
[39] Ann. d. l'Inst. Pasteur, 1900, 14, p. 415.
[40] Ztschr. f. Hyg. u. Infektionskr., 1907, 56, p. 1.
[41] Jour. Path. and Bacteriol., 1903, 8, p. 276.
[42] Comp. rend. Soc. de biol., 1912, 72, p. 13.

been abundant in the liver (Litch and Meyer[43]). This definitely proves that the presence of micro-organisms in the liver is not necessarily followed by an invasion of the bile.

Since Blachstein[7] found that rabbits, which have been given intravenous injections of atoxic strains of B. coli or B. typhosus, yield cultures from the bile for many days or weeks subsequently, numerous workers have conducted similar experiments. The observations on rabbits by Miyake,[44] Adrian,[45] Doerr,[1] Forster,[46] Lemiérre and Abraham,[47] Chiarolanza,[48] Conradi,[49] E. Blumenthal,[2] Morgan,[50] Tanabe and Takeuchi,[51] Arima,[52] Bully,[53] Perussia,[54] Johnston,[55] Hailer and Rimpau,[5] Gay and Claypole,[56] Nichols,[57] Weinfurther,[58] Gibson,[59] Lentz, Hailer and Wolf,[60] Besredka,[61] Flu,[62] and others with typhoid-paratyphoid and dysentery bacili; Baroni and Ceaparu,[63] Cano,[64] Schöbl,[21] and Creig,[65] with Vibrio cholerae; J. Koch[66] with staphylococci and streptococci; and Rosenow,[67] Ophüls and Smith,[68] with streptococci, indicate that intravenously inoculated bacteria must reach the gallbladder quite frequently and produce a distinct cholecystitis. It even has been demonstrated by Fränkel and Much,[69] and by

[43] Jour. Infect. Dis., 1921, 28, p. 27.
[44] Mitt. a. d. Grenzgeb. d. Med. u. Chir., 1900, 6, p. 479.
[45] Ibid., 1901, 7, p. 407.
[46] München. med. Wchnschr., 1905, 52, p. 1473.
[47] Compt. rend. Soc. de biol., 1907, 63, p. 252.
[48] Ztschr. f. Hyg. u. Infektionskrankh., 1909, 62, p. 11.
[49] Ztschr. f. Immunitätsforsch. u. exper. Therap., 1910, 7, p. 158.
[50] Jour. Hygiene, 1911, 11, p. 202.
[51] Mitt. a. d. Mediz. Gesellsch. z. Osaka, 1910, 9, refer.; Centralbl. f. Bakteriol. I. Ref., 1911, 50, p. 294.
[52] Arch. f. Hyg., 1911, 73, p. 265; Centralbl. f. Bakeriol., I, 1912, 63, p. 424.
[53] Ztschr. f. Hyg. u. Infektionskrankh., 1911, 69, p. 29.
[54] Pathologica, 1912, 4, p. 141.
[55] Jour. Med. Res., 1917, 37, p. 189.
[56] Arch. Int. Med., 1913, 12, p. 616.
[57] Jour. Exper. Med., 1914, 20, p. 573, 1916, 24, p. 497.
[58] Centralbl. f. Bakteriol., 1914-15, 75, p. 379.
[59] Jour. Roy. Army Med. Corps, London, 1917, 29, p. 601.
[60] Arb. a. d. Reichs Gsndhsamte, 1918, 51, p. 1.
[61] Ann. de l'Inst. Pasteur, 1919, 33, pp. 557 and 301.
[62] Geneesk. Tijdschr. v. Nederl. Indie, 1918, 58, p. 67.
[63] Compt. rend. Soc. de biol., 1912, 72, p. 894.
[64] Centralbl. f. Bakteriol., 1913, 72, p. 124.
[65] Indian Jour. Med. Res., 1913-14, 1, pp. 44 and 59; 1914-15, 2, pp. 1, 28, and 907; 1915-16, 3, pp. 259 and 397; 1917, 4, pp. 651 and 658; 1917-18, 5, pp. 81 and 89.
[66] Ztschr. f. Hyg. u. Infektionskrankh., 1908, 60, p. 335; 1908-09, 62, p. 1; 1911, 69, p. 436.
[67] Jour. Infect. Dis., 1916, 14, p. 527.
[68] Proc. Soc Exper. Biol. and Med., 1918, 15, p. 113.
[69] Ztschr. f. Hyg. u. Infektionskrankh., 1911, 69, p. 342.

E. Fränkel,[70] that paratyphoid B or A bacilli may exhibit selective properties whereby they tend to realize in the gallbladder and bile of animals infected by intraperitoneal injections or by feeding. Most of the workers mentioned agree that the bacteria reach the gallbladder in the bile secreted by the liver. In some instances (F. Blumenthal and Nichols) they may arrive in from 5 to 10 minutes. The bile capillaries apparently receive the organisms from the blood of the interlobular veins, the endothelial lining being the only barrier which separates the biliary system from the blood stream. The following questions naturally arise: 1. Is this intrahepatic passage of micro-organisms from the blood to the bile a normal secretory function? 2. Is it a mechanical process? 3. Does this elimination follow the pathologic changes which have been produced in the blood vessel wall by the bacterial toxins? Neither Sherrington nor J. Koch, both careful workers, believe in a true physiologic elimination, but they agree that a transit of bacteria across the hepatic membranes can occur without the detectable presence of blood in the infected bile. It is furthermore stated by Sherrington that the membrane may remain normal, unruptured and impervious to blood cells, while the soluble toxin of the bacteria may injure the barrier sufficiently to cause a slight inflammation which is followed by the passage of the accumulated micro-organisms. His view is well supported by the following facts: First, in his experiments nonpathogenic bacteria never appeared in the bile. Second, J. Koch,[71] failed to produce cholecystitis with avirulent skin staphylococci, injected in small doses. Furthermore, Wyssokowitch,[72] and also Blachstein considered the liver necroses prerequisites for the infection of the biliary secretions. It has been pointed out in the preceding paper (IV), confirming the observations of Weinfurter and Nichols, that the intravenous injection of typhoid bacilli produces bile invasion or infection only when large doses of recently isolated strains are employed. Dosage, virulence and lesions and not the secretory, detoxifying activity of the liver, are the prerequisites for the passage of bacteria from the blood to the bile capillaries. In the light of these established facts the conception of a purely mechanical passage deserves little consideration. It is most unlikely that the masses of bacteria, which are thrown into the liver capillaries, cause "permeability of the liver filter," by rupturing the

[70] München. med. Wchnschr., 1918, 65, p. 413.
[71] Ztschr. f. Hyg. u. Infektionskrankh., 1908, 60, p. 335.
[72] Ibid., 1886, 1, p. 3.

capillary wall and by being flushed with the liver secretion into the biliary capillaries. Our experiments, to be reported later, indicate that the transit of bacteria from the blood to the bile is governed by the endothelial lining of the blood capillaries.

The analysis of the various routes by which bacteria reach the gallbladder does not exhaust all the possibilities. Particularly in connection with the typhoid gallbladder problem, there has arisen a controversy of considerable interest. The descending hemato-hepatogenous passage of typhoid bacilli, as described in the foregoing, was accepted as the most likely mode of bile invasion until J. Koch and Chiarolanza [48] claimed to have proved that the bile may also become infected through the capillaries of the gallbladder wall. Koch drew his conclusion from the histologic picture in a human case of typhoid cholecystitis, in which he found nests of typhoid bacilli lying in close relation to the capillaries of the folds of the mucous membrane. Lange and Roos,[20] Creig,[73] and others (Posselt [74]) have more or less accepted this view. At Koch's suggestion Chiarolanza tied the cystic duct of rabbits in two places, and injected immediately following the operation typhoid bacilli intravenously. He recovered them from the gallbladder 24 to 48 hours later. These experiments have been justly criticized by E. Blumenthal and Nichols. A careful scrutiny of the protocols published by Chiarolanza indicates that the bile was bloody even in animals examined in less than 24 hours. Technically, the experiments have been poorly executed; he admits that in the majority of incidences the entire gallbladder has been necrotic. The illustration in Figs. 6 and 7 of his article, which show bacterial emboli in the wall, supply ample proof for this statement. In ligating the cystic duct the accompanying cystic artery was in all probability also tied and hemorrhagic infarction occurred from incomplete collateral circulation.

Those who attempt to refute the general conception of an embolic wall infection, depend for their argument on certain experiments conducted by R. Doerr.[1] This worker also tied the cystic duct, but waited from 3 to 5 days before giving the intravenous injection. Under these conditions no infection occurred. The conclusions drawn from these experiments are for the following reasons invalidated: First, no cultures were made of the gallbladder wall in which a focus of infection may be present from 24 to 72 hours before the bacteria break

[73] Indian Jour. Med. Res., 1914-15, 2, pp. 1, 28 and 907.
[74] Ergebn. d. allg. Path. u. path. Anat., 1919, 19, pp. 351 and 471.

through the epithelium leading to contamination of the cystic bile. Second, only one experiment is reported, and in this no mention is made of the number of animals in which either one-half or one loopful of typhoid culture produced a gallbladder infection. The impression is gained that for the successful experiments at least two loopfuls of culture were injected. In the light of these inconsistencies we feel that the conception of an arterial invasion of the gallbladder in rabbits inoculated with large doses of typhoid bacilli is neither proved nor disproved. Experiments and histologic studies to be reported in this paper make it certain that in addition to the common route, namely, the hemato-hepatogenous one, the transverse route via capillaries of the gallbladder wall may be responsible for the development of an experimental cholecystitis. Observations collected from nearly 500 necropsy examinations on rabbits, the results of which are recorded in paper IV in connection with the immunity experiments, and some cases of spontaneous rabbit paratyphoid have materially strengthened this conclusion. Moreover, it has already been proved by Rosenow,[67] that streptococci can invade the gallbladder wall in the form of emboli. The work of this writer leads, however, to a consideration of the last possibility by which bacteria can reach the biliary tract, namely, the lymphatics. From an experimental standpoint this route is of no importance. It is possible that the observations of Ledingham (see Morgan [50]), who noted typhoid bacilli in the gallbladder of guinea-pigs that had been injected intraperitoneally, can be explained on this basis. Clinically, it is an established fact that in the course of peritonitis or other abdominal infections (appendicitis) streptococci can be transported through the lymphatics to the biliary passages. The lymph vessels of the pancreas, periduodenal and peripyloric tissues, end in the fossa transversa of the liver; infection of the extra hepatic biliary system from these regions is therefore quite possible (Mix [75]). In a number of typhoid experiments we observed the frequent secondary infection of the gallbladder of rabbits with streptococci. In these cases the bile was sterile, while the wall gave on proper cultivation an abundant growth of indifferent streptococci. Coinciding with the observations of numerous surgeons (Baron [76]), we also noted in chronic cholecystitis of animals the displacement of B. typhosus by B. coli or by streptococci. A lymphatic invasion is the most likely route of these infections. The fact that the lymphatic spaces and vessels of the rabbit's gallbladder

[75] Illinois Med. Jour., 1914, 25, p. 17.
[76] Beitr. z. klin. Chir., 1912, 77, p. 447.

or the extrahepatic ducts are seriously injured as a result of bacterial growth in the cystic bile or in consequence of mechanical injury to the biliary system lends support to this view. The so-called ascending route of infection is in all probability due to an invasion of bacteria through the lymphatics. The experiments of M. Müller,[77] with the typhoid bacillus in mice, moreover suggest that the spleen, liver, and their adnexa can readily become infected by way of the lymph stream. In animals with a native immunity against the micro-organisms this mode of invasion is the rule.

In summarizing these general considerations, it must be concluded that for the experimental production of a cholecystitis with intestinal organisms, such as the typhoid-dysentery bacilli and cholera vibrios, the hemato-hepatogenous route is the only one which has been definitely proved. An infection through embolic invasion of the wall may occasionally occur. This route demands, however, further experimental proof. An ascending infection follows, as a rule, the lymphatics; secondary or superimposed infections with streptococci take place through the same channels. The mechanism of bacterial transit from the interlobular veins or branches of the hepatic artery to the bile capillaries, and the factors which lead to a persistence of disappearance of the eliminated bacteria in the cystic bile and gallbladder, are as yet unknown. The facts presented later were collected with the desire to solve some of the questions which suggested themselves in the course of the critical analysis of our present knowledge concerning the problem of experimental typhoid cholecystitis in laboratory animals.

OPERATIVE PROCEDURES AND METHOD OF COLLECTING HEPATIC DUCT BILE FROM LABORATORY ANIMALS

For the experiments to be recorded in this and subsequent papers the sterility of the bile samples to be tested is of utmost importance; it is therefore self-explanatory that the operative procedures are conducted under strict asepsis. All animals used are completely etherized. To facilitate the exposure of the hepatic duct a thick pad is placed under the vertebral column between the costal and lumbar vertebrae. The shaved skin is thoroughly cleansed with soap and water, alcohol, ether, and painted with tincture of iodine. The body of the animal is always

[77] Ztschr. f. Fleisch. u. Milchhyg., 1911-12, 22, p. 106; Centralbl. f. Bakteriol., I, 1912, 62, p. 335.

covered by sterile linen sheets and the field of operation is blocked by sterile towels, as customary for laparotomies.

As a rule, a medium incision not longer than 5 cm. is made, extending from the xiphoid cartilage to the umbilicus. Occasionally in rabbits the viscera are exposed by a transverse incision extending along the rib margin of the right side. The latter method has, however, no advantage over the median incision. After the peritoneum is opened the left hand of the operator grasps with three fingers the pyloric region of the stomach and by bringing this portion gently into the opening of the abdomen, the hepatic duct is made visible. Particular care is necessary that during this act of the operation no mesenteric blood vessels are torn. The common duct is made accessible by fixing the small intestines with gauze pads soaked in warm saline. The mesentery of the duct is cut, avoiding the blood vessels, and a threaded French needle passed under the duct about 0.5 cm. from the duodenum. The tied thread enables the assistant to put the duct on the stretch. A second silk thread is then placed about 1 to 1.5 cm. from the first ligature; the duct is transversely incised with a small pair of sharp scissors and usually without any difficulty a glass cannula with a good neck can be inserted. The thread is then tied on the neck of the tube. When the cannula is properly placed, perfectly clear, slightly yellowish-green bile enters it as soon as the first ligature which holds the duct on a stretch is released. The gall cannula is connected by a fairly stiff rubber tubing, which is brought out either through the abdominal incision or through a stab wound on the right side of the abdominal wall. A series of silk sutures close the peritoneal cavity. The wound is covered with collodium.

The animal is then fixed on a padded and electrically warmed board. The rubber tube is connected with a sterile glass tube in a two-holed stopper, the latter being inserted in a pyrex test tube or an alkaline-free graduated centrifuge tube. The glass and rubber connections hold about 0.8 to 1.0 c c of bile.

The operation is easily performed on rabbits, and provided properly made cannulas are used, not only contamination but also admixture of blood is regularly avoided. Of about 80 rabbits operated on, with the technic and the aseptic precautions mentioned, one or two bile samples only revealed contaminating staphylo- or streptococci. In guinea-pigs, the technic of exposing the hepatic duct without hemorrhage is considerably more difficult, and blood-thinned bile specimens occurred

in about 10% of the 30 animals operated on. As will be shown later, rats can be successfully prepared for hepatic bile collection, but the rate of flow is so slow that the animals operated on have to be kept in a fixed position for more than 12 hours. This prolonged period of collection produced in the two instances attempted, specimens contaminated by staphylococci.

The preparation of hepatic duct fistulas in dogs, cats, monkeys and goats offered no difficulties and in every instance sterile samples were obtained. It was, however, noted that the manipulations of the duodenum and of the liver caused regularly a prolonged period of reflex which inhibited bile secretion. With most of our dogs and cats we failed to obtain the necessary amount of liver bile without the use of cholagogues given either in form of ox bile by stomach tube 2 hours before the operation or more advantageously by injecting intravenously from 1 to 2 gm. of sodium taurocholate in warm saline solution. The cholagogue effect lasted usually from 2 to 3 hours; prolonged experimentation necessitated repeated injections. We are conscious of the fact that such procedures materially altered the constituents of the secretion, as will be shown later. The observations to be recorded on these biles should be viewed from this standpoint. The late Miss Foster of this laboratory has shown that about 90% of the injected taurocholic acid is eliminated in the fistular bile during the 2 to 4 hours following its injection. Numerous attempts to use bile obtained from dogs with simple gallbladder fistula were unsuccessful. The samples were regularly so badly contaminated with various micro-organisms that, in order to prepare suitable test specimens, repeated heating was necessary. The study of such samples naturally introduced new factors, which were not in the scope of our inquiry, and which dealt primarily with the bile freshly secreted from the liver.

In a number of rabbits the cystic duct was doubly ligated and cut before placing the cannula in the common duct. This operation is admittedly very delicate; utmost care is necessary to avoid injury or ligature of the cystic artery. The majority of our attempts resulted in hemorrhagic infarction, or minor circulatory disturbances in the veins of the gallbladder wall, which either became totally necrotic or showed hemorrhages and escape of blood into the lumen of the viscus. It is obvious that the injured tissues offer an excellent opportunity for localization of the intravenously inoculated typhoid bacilli. Our observations on the embolic invasion of the capillaries of the gallbladder

mucosa were made on rabbits, which were neither laparotomized nor exposed to injury by ligation of the cystic duct or common duct.

The selection of the rabbits for the experiments to be reported followed the principles outlined in previous papers. If possible, members of the same litter were chosen. In about 1% of the rabbits and guinea-pigs employed spontaneous cholecystitis either due to B. coli (2) or streptococci (3) staphylococci (1) impaired the value of the experiment. The cystic bile was in these instances either distinctly changed in color or sections of the thickened wall revealed a diffuse lymphocytic infiltration of the mucosa and submucosa. Occasionally the bile appeared to be normal, while the histologic picture exhibited a low grade infection of the lymphatics of the mucosa and subserosa. The preparation of the suspension of typhoid bacilli to be injected, the cultivation of the bile and blood specimens and the numerical determination of the bacteria in the tissues were treated in the same manner as stated in previous papers.

EXPERIMENTAL DATA

From the discussion of the present knowledge concerning the possible path by which the typhoid bacilli on intravenous inoculation reach the gallbladder, it is evident that the hemato-hepatogenous system is probably the usual route. The factors which favor the transit of the bacteria from the blood to the bile are, however, incompletely investigated. Moreover, nothing is known relative to the exact number of typhoid bacilli, which enter the biliary system in this manner, and their fate in the biliary secretion. The experiments thus far published (with the exception of those by Nichols) deal with the phase of the problem only in a qualitative manner. We are by no means convinced that the mere presence of bacteria in the bile, after an intravenous injection of typhoid bacilli in a sufficiently large number to ensure passage into the excretion, leads always to a cholecystitis or even to a temporary persistence of the organisms somewhat analogous to the human carrier state. Furthermore, one should know how soon after the injection and for what period of time the bacilli are discharged in the bile. In the preceding paper the fate of the typhoid bacilli was determined by cultivating the tissue from 1 to 160 hours after the injection. No attention was paid at that time to the behavior of the bacilli in the blood stream during the first hour. Bull [78] and others have already considered

[78] Jour. Exper. Med., 1914, 20, p. 237; 1915, 22, pp. 475 and 487; 1916, 23, p. 419; 24, p. 25.

this phase of the problem, but in connection with the study of the disappearance of the leukocytes from the peripheral blood stream of animals with biliary fistulas that had been intravenously inoculated with living typhoid bacilli, the fate of these organisms in the blood vessels was simultaneously investigated.

The work of Nichols suggested a relationship between immunization and elimination of the injected bacteria. In the experimental study of this phase the results of this writer have in part been confirmed and amplified by our work on guinea-pigs.

THE ELIMINATION OF TYPHOID BACILLI IN THE HEPATIC DUCT BILE OF NORMAL AND IMMUNIZED RABBITS, GUINEA-PIGS AND DOGS

Charts 1 and 2 illustrate the rate of elimination of typhoid bacilli in the hepatic duct bile collected periodically from common duct fistulas of 2 normal and 2 immunized rabbits. The degree of leukopenia and the rate of disappearance of the inoculated bacilli are also demonstrated. The observations have been collected from a series of 8 successful experiments on coccidiosis-free animals. The cystic duct has always been tied before placing the hepatic duct cannula, and the animals have been permitted to recover from the operation (at least 2 to 3 hours) before the bacteria have been inoculated. Varying amounts of cultures grown on agar have been introduced intravenously. When the number of bacteria injected has been less than 1,000 million, the plates prepared from the bile specimen collected for a period of 2 hours have never shown more than 10 bacteria (table 1).

TABLE 1

ELIMINATION OF TYPHOID BACILLI IN HEPATIC DUCT BILE IN IMMUNIZED AND NORMAL RABBITS

Experiment	Rabbit	Number of Typhoid Bacilli Inoculated	Total Colonies in Bile	Time of Collection
1	1114a Immunized	600,000,000	6 in 21.9 c c	120 Min.
	1114b Normal	600,000,000	7 in 24.1 c c	120 Min.
2	1147a Immunized	10,000 million	12 in 20.35 c c	120 Min.
	1147b Normal	10,000 million	28 in 25.9 c c	120 Min.
3	1141a Immunized	12,000 million	13 in 15.3 c c	60 Min.
	1141b Normal	12,000 million	183 in 15.6 c c	60 Min.
4	1148a Immunized	20,000 million	23 in 7.4 c c	120 Min.
	1148b Normal	20,000 million	422 in 10.7 c c	120 Min.
5	1136a Immunized, 1½ year old	24,000 million	3,377 in 36.6 c c	120 Min.
	1136b Normal, 1½ year old	24,000 million	24,609 in 27.0 c c	120 Min.

Inoculations of large doses varying from 10,000 to 24,000 million produce a rapid discharge of typhoid bacilli. In one instance it was profuse. With the exception of exper. 2, the general character of the

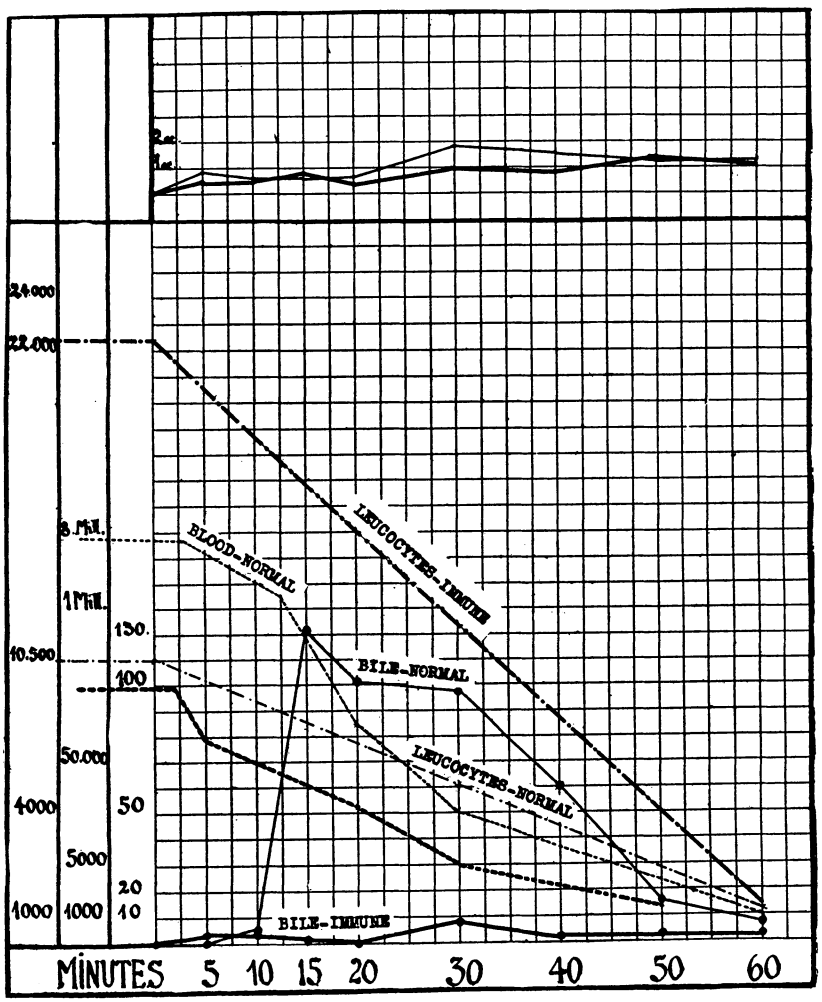

Chart 1.—Rate of elimination of typhoid bacilli in hepatic duct bile in 1 normal and 1 immunized rabbit. . Degree of leukopenia and disappearance of bacilli from bloodstream. Rate of bile flow during the experiment.

curve of elimination has followed the one given in chart 1. In the normal animal the bacilli appear in greater numbers than in the immune. There are some differences in elimination characteristics for certain

litters, depending on the age of the individual animals. The age of the culture, whether grown with or without rabbit blood, has no influence on the total number of bacilli discharged in the bile. In every instance but one the cystic bile has been sterile.

As a rule, the elimination of the bacilli is immediate. Making an allowance for the bile which is present in the cannula at the time of the intravenous injection, it is obvious that less than 5 minutes elapse before the bacteria reach the biliary passages. Moreover, the maximum discharge occurs in the first 5 to 15 minutes, in some instances even in less time. In subsequent periods the number decreases rapidly and not infrequently ceases completely at the end of 1 hour. While these observations confirm the facts already reported by v. Fütterer, Biedl and Kraus, Blumenthal and Nichols, in very exceptional cases, as has been exemplified in the data published by Doerr, and by ourselves (paper IV), the elimination is delayed or even on repeated inoculation is not demonstrable. According to one of Doerr's tables (footnote 1, page 629), at least 8 hours elapsed before the typhoid bacilli inoculated in an amount of 2 standard loopfuls, an average of 5,000 million organisms, were present in the cystic bile of rabbits. Three of his animals in the same series killed on the 2nd, 4th, and 6th hours gave sterile cystic bile specimens. Doerr has failed to state whether the secretions have only been plated or whether they have also been enriched. On several occasions it has been noted that even repeated inoculations of large doses of typhoid bacilli have resulted either in sterile hepatic duct bile specimens, or the number of organisms demonstrated by plating has been below 10 and has been insignificant in proportion to the inoculum. This statement is best illustrated by the presentation of an experiment.

Exper. 9.—Normal rabbit 1059 (weight 2,525 gm.) was inoculated with 7,750,000,000 typhoid bacilli (polyhomogenous 5 strains) after his cystic duct had been ligated and a common duct fistula placed. During the initial period of 60 minutes no bacilli were discharged. At the end of the first hour an additional inoculation of 16,000,000,000 bacteria was made and the collection of the samples continued for 60 minutes. A total of 7 colonies was counted in specimens obtained 100, 110 and 120 minutes after the first injection. The collecting tube contained 2 organisms, while 0.8 c c of deep green, blood-free cystic bile gave 396 colonies. The gallbladder wall registered 23,000 typhoid colonies per 100 mg. of tissue.

The leukocytes dropped from 18,000 to 1,300 per c c, and the blood freed itself of the bacteria as follows: 1 minute after the 1st injection, 1,400,000 per c c; 60 minutes after the injection, 30,000 per c c; 1 minute after the 2nd injection, 19,000,000 per c c; 60 minutes after the injection, 660,000 per c c in the peripheral blood and 100 c c in the heart blood.

It is evident from this and similar experiments that (1) even a large dose of typhoid bacilli in a limited number of rabbits never leads to a discharge of the bacilli by way of the hemato-hepatogenous route; (2) the gallbladder bile may become infected even after ligation of the cystic duct; and (3) particularly if one considers the tremendous dosage and the rapidity with which the blood stream freed itself of organisms, the transit of typhoid bacilli in the liver from the blood to

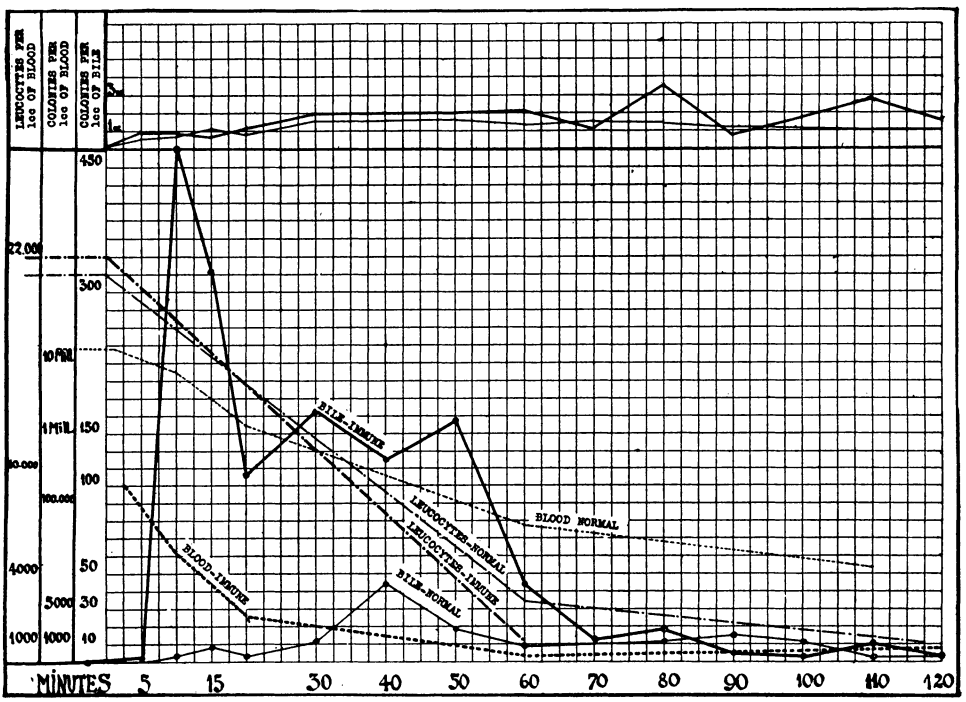

Chart 2.—Elimination of typhoid bacilli in the duct bile of a normal and of a recently immunized rabbit.

the bile capillaries in the first 2 hours after the injection cannot be the outcome of a mechanical rupture of the capillary walls by the masses of bacteria resulting in a flushing of the organisms into the biliary secretion.

Additional experiments confirm the foregoing conclusions as far as they concern the infection of the gallbladder whose cystic duct has previously been ligated. Inoculations of large doses of typhoid bacilli may cause an infection of the cystic bile in animals with a ligated cystic duct. Our experiments were originally conducted according to the procedure of Doerr. Nine rabbits were operated on; the cystic duct was carefully tied; and the animals permitted to

recover from the laparotomy. After a lapse of from 4 to 6 days, they were inoculated with a sublethal dose of B. typhosus (varying from 2 to 4,000 million). Twenty-four to 72 hours after the injection, the animals were killed and their tissues cultivated. Five animals must be eliminated from consideration on account of the profound changes in the gallbladder wall (hemorrhages, partial necrosis, etc.). Three of these rabbits had sterile cystic biles while the remaining 2 showed varying numbers of typhoid bacilli. Four rabbits were successfully operated on; the bile at necropsy was deep olive green and

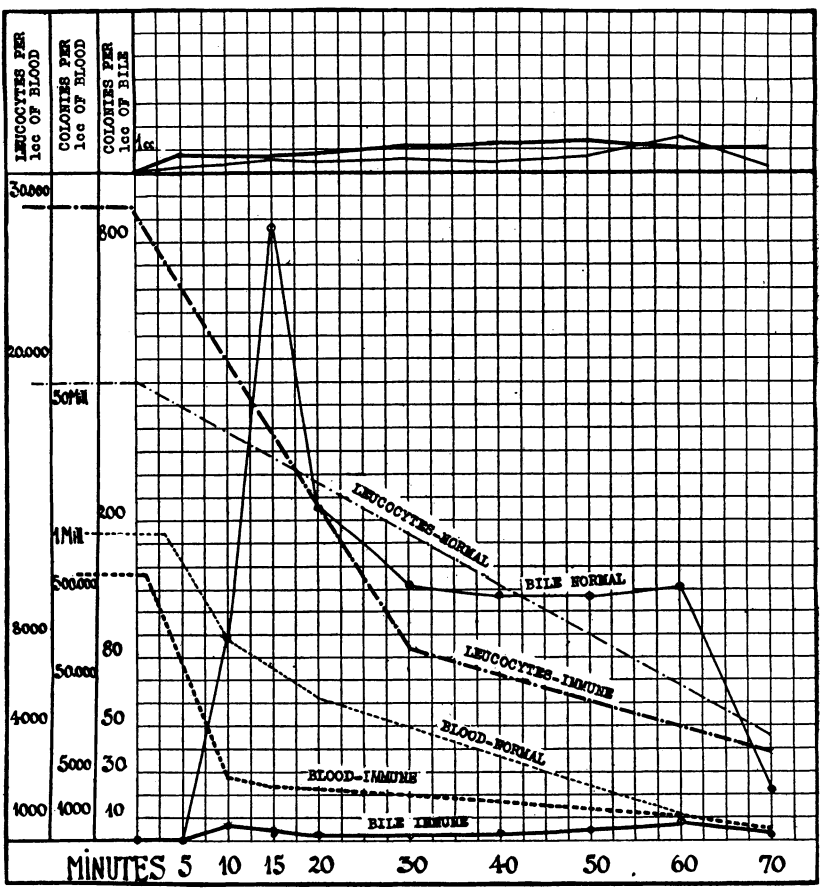

Chart 3.—Elimination of typhoid bacilli in the duct bile of a normal and of an immunized guinea-pig.

viscid; the gallbladder wall was microscopically intact. Neither of these animals gave a positive cystic bile culture and the gallbladder wall was either sterile or contained only a few bacilli. Moreover, the duodenal content and mucosa of these rabbits was sterile or failed to give specific colonies. On first consideration these experiments seem to confirm the experiences of Doerr

and Blumenthal, but on further comparison with other data at our disposal considerable hesitancy is felt in drawing far-reaching conclusions. The absence of typhoid bacilli in the duodenum deserves in this connection some consideration. Laparotomized rabbits succumb readily to a typhoid intoxication in the first 12 to 24 hours, as has been stated repeatedly. It is technically impossible to infect such animals with recently isolated typhoid strains in amounts which ensure elimination in the hepatic duct bile and which lead to an invasion of the gallbladder wall. In numerous experiments on rabbits which were not previously operated on it was noted that only 40% of the animals developed infected gallbladders, when injected with the same technic as in the above tests. The experimental data recorded in animals previously laparotomized are therefore seriously invalidated and cannot be used in any argument against the theory of embolic wall infection. Exper. 9, previously described, suggests a means of overcoming these technical difficulties. In these experiments a number of rabbits were laparotomized, the cystic duct carefully dissected, ligated and cut, and a common duct cannula inserted. After a lapse of from 3 to 5 hours, they were injected intravenously with large amounts of typhoid bacilli (15 to 24 billion, one slant of peptic digest of ½ slant of rabbit-blood agar cultures). Bile specimens were collected for from 2 to 8 hours, and at the end of this period the animals were killed. Careful cultures revealed in 2 of the 6 rabbits successfully operated on, 69 and 117 typhoid colonies in 0.4 and 0.7 c c, respectively, of deep green bile. Neither of the 2 specimens contained traces of blood; the gallbladder walls gave 8,700 and 6,900 colonies, respectively, per 100 mg. of tissue. During the observation period of 8 hours, 3,300 and 1,280 typhoid bacilli were eliminated by the liver. These experiments suggest that in a number of rabbits typhoid bacilli can reach the cystic bile by way of the blood vessels of the gallbladder wall provided a sufficiently large inoculation is given. The manner in which the bacilli enter the bile will be discussed in connection with the consideration of the microscopic findings in these gallbladders.

It is definitely shown in chart 1 and table 1 that the immunized rabbit discharges less organisms than the normal animal. This fact is even more strikingly shown in chart 3 illustrating the same type of experiment in a normal and immunized guinea-pig. The graph has been prepared from the data of an experiment selected from a series of 4. The study conclusively proves that in the immunized guinea-pig either no bacteria or few pass into the biliary capillaries. The result depends entirely on the number of bacilli inoculated, as is shown by the following figures:

TABLE 2

NUMBER OF BACTERIA IN HEPATIC DUCT BILE OF IMMUNIZED AND NORMAL GUINEA-PIGS AFTER INOCULATION OF TYPHOID BACILLI

B. Typhosus Injected	Time after Injection	Immunized	Normal
100,000,000	120 Minutes	0	2,216
300,000,000	60 Minutes	11	1,942
450,000,000	60 Minutes	33	239
600,000,000	60 Minutes	45	1,680

As the immunized guinea-pigs can destroy typhoid bacilli more readily than the normal animal, a difference which is not demonstrable in the rabbit, it is not at all surprising to find the contrast we have noted in the elimination of bacteria. Immunization obviously produces in the blood vessels of the liver some factors controlling the elimination of the organisms which can only be overcome by a large inoculation. The nature of these factors is not definitely established, but we have been able to demonstrate in the guinea-pig histologically a very active phagocytic action of the endothelial cells. At the end of 2 hours most of the Kupffer cells are packed with typhoid bacilli. The same phenomenon occurs also in the rabbit but to a less marked degree. The endothelial lining of the liver capillaries, which is endowed with the maximum ability to phagocytize and to destroy typhoid bacilli, will also be resistant to the typhoid toxins, which tend to injure the capillary wall and permit in this manner an escape of some bacteria into the adjacent biliary capillaries. The guinea-pig is relatively insusceptible to the typhoid toxin [79] present in the bacterial emulsion. The maximum elimination of the bacteria in the hepatic duct bile occurs in the first 10 to 25 minutes after the injection, corresponding to the time when the liver has accumulated approximately from 25 to 40% of the bacterial masses inoculated. In this connection one recalls the observations of Helly [80] and of Schwarz,[81] who have found typhoid bacilli not only inside, but outside of the capillaries in the pulp cords and the malpighian bodies of the spleens of guinea-pigs 10 minutes after an intravenous injection. The capillary system in general, and not only that of the liver vessels, is therefore permeable to bacteria shortly after a drastic intravenous injection. In the immunized guinea-pigs polymorphonuclear and endothelial phagocytosis begins immediately, and comparatively few organisms can be found free in the blood stream. In accordance with this fact, the number of typhoid bacilli per c c of peripheral blood of the immunized guinea-pig is less in the first 20 minutes than in the normal animal. As long as the bacterial masses to be phagocytized are comparatively small and can be handled by the cells destined for this purpose, no bacteria will enter the bile. In case the injection and the subsequent hepatic invasion are overwhelming, a transit of organisms will be recorded. The elimination is apparently

[79] For a detailed consideration of the so-called typhoid toxin consult the recent summary of H. Zinsser, Jour. Immunol., 1902, 5. p. 265.

[80] Beitr. z. path. Anat. u. z. allg. Path., 1903, 34, p. 387.

[81] Ztschr. f. Heilk., 1905, 26, p. 295.

not an excretory process. This statement is based on the following observation. The foregoing experiments were repeated after adding to the bacterial suspension a 1% Congo-red solution in saline (1 c c in guinea-pigs and 10 c c in rabbits, Cecil and Weil[82]). The dye appeared in the normal and immunized animal in from 4 to 18 minutes. No differences in the amounts of the stain excreted could be determined in the 2 animals, but numerous typhoid bacilli were present in the bile collected from the normal, while sterile plates were obtained from the immunized guinea-pigs. In some instances the excretion of the dye preceded the appearance of bacteria in the bile by from 8 to 15 minutes. The results just mentioned suggest that a further study of the transit of bacteria from the blood to the bile capillaries be combined with an analysis of the excretory function of the liver of the same animal by some similar test. All these observations constitute a strong argument in support of the conception of a cellular immunity, particularly when tests are conducted on guinea-pigs which have been vaccinated several months previous to the tests and which have lost their serum immune bodies. The number of such experiments is relatively small, but definitely indicates that a guinea-pig once treated with typhoid bacilli will always behave like an immunized animal even when the usual tests suggest that his immunity has been lost. A persistent experimental typhoid cholecystitis can only be produced in immunized guinea-pigs with very heavy intravenous infections.

In the rabbit the endothelial cells of the liver capillaries function less vigorously than in the guinea-pig, but differences in activity of the cells of the normal and of the immunized animals can be detected by the bile elimination test, provided a sufficient time has elapsed between the last immunizing dose and the test injection. Immunized rabbits injected with small doses of typhoid bacilli on the 6th (chart 2) or 10th day (Nichols, footnote 27, 504) discharge more bacteria than normal ones. The endothelial barrier is apparently imperfect; the cells have in consequence of the vaccination not sufficiently regenerated or acquired the function of "immunity," and bacteria leak in to the bile capillaries. The observation has been confirmed repeatedly. Irrespective of the agglutinin content of the blood or the number of injections, the bile of immunized rabbits infected before the 6th to 10th day contains more bacilli than the same secretion of normal rabbits.

[82] Jour. Am. Med. Assn., 1917, 69, p. 521.

For example, rabbit 1374 was immunized intravenously by 10 inoculations of heat-killed typhoid bacilli. Six days after the last injection, when the agglutination titer was 1:200,000, the animal, together with rabbit 1090 (a "recovered carrier" with an agglutination titer of 1:200) was inoculated with 8.5 billion living typhoid bacilli. Killed after a lapse of 2 hours, the bile of rabbit 1374 contained 800,000 typhoid bacilli per 3.3 c c, while 1.2 c c bile of rabbit 1090 proved sterile on direct plating and 0.4 c c of the same on enrichment in broth was likewise sterile. The destruction of the bacteria in the tissues of both animals was practically identical. The blood contained 200 and 300 typhoid bacilli per 1 c c of blood.

Our experiments fully explain one observation made by Nichols. One of us (K. F. M.) immunized the rabbit used by this writer. According to the available records, the test injection was given on the 5th day after the last immunizing inoculation. The conclusion of Nichols, that more bacilli appear in the bile after an injection of the same dose in immunized animals than in normal animals, deserves some modification. It should read "immunized animals which have not recovered from the treatment discharge more bacilli." The facts elucidated by the experiments may have some bearing on the observations of Metchnikoff and Besredka,[83] that anthropoid apes are not protected against an alimentary typhoid infection when the animals are tested in from 4 to 6 days after finishing the immunizing treatment. They partially explain our own experiments reported in paper III: namely, immunized rabbits infected on the 10th day after the injection of the last dose of vaccine develop in a higher percentage of instances gallbladder infections than nonprotected animals. It is not unlikely that the reports of Creig[84] and Flu[62] on rabbits developing gallbladder infections in the course of immunization with cholera-like vibrios and Flexner dysentery bacilli may in part be explained by the foregoing fiindings. The question naturally arises, what constitutes a safe period for recovery? Until recently, all processes of immunity have been gaged by the appearance or disappearance of immune bodies in the blood serum, while little or no attention has been paid to the reparative changes which by necessity must take place in the injured tissues. Nobody will deny that heat-killed typhoid vaccines produce, for example, liver necrosis, proliferation of macrophages, etc., but how many days are necessary to cause a restitutio ad integrum fails to interest the serologist, who studies disease and infection only by test-tube experiments. It is a matter of common knowledge in our laboratory that

[83] Ann. d. IInst. Pasteur, 1911, 25, p. 193.
[84] Indian Jour. Med. Res., 1915-16, 3, pp. 259 and 397.

a rapid method of immunization, for example, the procedure suggested by Fornet and Müller, by Bull [85] and by others, produces immune serums of great potency with no loss in animals and no gallbladder infections. On the other hand, the "standard method" of immunization, at 7 to 10 days, in comparison furnished poor serums, frequent losses of animals from cachexia and numerous gallbladder infections. The rapid method of immunization of rabbits finds, therefore, some advantageous justification in view of these data.

The elimination of typhoid bacilli in the hepatic duct bile of dogs has been studied in 10 animals. The results have been inconstant. Differences between the rate and degree of bacterial elimination in immunized and normal dogs have been indefinite. Injections of less than 8,000 million have failed to cause transit of bacteria, in dogs varying in weight from 14 to 26 pounds. Cats behave in a similar manner. It is generally known from the studies of Cushing [86] and our own, that typhoid bacilli introduced directly into the cystic bile of dogs disappear rapidly and that a cholecystitis can only be produced by considerable injury of the wall or by placing a foreign body in the gallbladder (Marxer [16]). A more careful study of the mechanism of gallbladder infection in these species of animals has therefore been considered superfluous.

THE DISAPPEARANCE OF THE TYPHOID BACILLI AND OF THE LEUKOCYTES FROM THE PERIPHERAL BLOOD STREAM

Blood samples were collected from the ear veins at regular intervals during the experiments on fistula animals. By means of pipettes with a capacity of 0.01 c c the blood drops collecting on a sterilized area of the ear were aspirated. Leukocyte counts were obtained from the same specimens. For each test a fresh incision was made. Hypocoagulability favored the collection of blood and as long as the blood pressure was normal, uncontaminated cultures were obtained. The findings are included in charts 1, 2 and 3.

In a general way, the observations confirm the well-known fact, originally established by Wyssokowitch [72] and von Fodor,[87] that bacteria intravenously inoculated disappear rapidly from the circulation. The analysis of the data presented in paper IV indicates that at least one

[85] Jour. Exper. Med., 1916, 24, p. 25.

[86] Bull. Johns Hopkins Hosp., 1899, 10, p. 166.

[87] Arch. f. Hyg., 1886, 4, p. 129; Von Fodor, I., and Rigler, E.: Centralbl. f. Bakteriol., I, 1898, 23, p. 930.

hour is necessary to cause a complete absence of the typhoid bacilli from the heart blood, obtained from living animals by puncture. Furthermore, it has been shown that the organisms are deposited in the liver, spleen, bone-marrow and lung. At the end of from 1 to 2 hours the bacteria may reappear and circulate in moderate numbers until the 4th to 8th day. In paratyphoid infected animals the period is even longer and in case the animals succumb the bacilli may be demonstrated in the blood in large numbers at the time of death. The disappearance of the typhoid bacilli is decidedly more rapid in the immunized animals, but not as complete as demonstrated by Bull. Our results differ from those reported by this writer for these reasons: (a) a heavier dose of bacteria was injected; and (b) the blood samples were collected from the peripheral vessels instead of from the heart.

The mechanism of the removal of bacteria which is the same even on repeated injections (exper. 9) has been the subject of considerable study. It is interpreted by Bull [88] to be a result of in vivo agglutination, but recently Debres and Govaerts [89] attribute the clumping of the intravascular bacteria to the action of the blood platelets. The intravenous inoculation in their opinion causes a disturbance of the plasma with an agglomeration of platelets, which in turn engulf the bacteria. The agglomerated masses vanish from the blood stream to be deposited subsequently in the capillaries, sinusoids, etc., of the organs. The removal of the blood platelets produces in turn a hypocoagulability. We have had occasion repeatedly to note the prolongation of the coagulation time of the blood collected 15 to 20 minutes after the inoculation of living or dead typhoid bacilli. The following facts also speak against an in vivo agglutination. According to Ten Broeck [90] and our own observations, virulent and invasive hog cholera or paratyphoid bacilli are rapidly clumped and disappear from the blood even though the blood contains no demonstrable agglutinins, while Hopkins and Parker [91] noted rapid removal of virulent hemolytic streptococci without any evidence of agglutination. In vivo agglutination may be positive even when in vitro experiments fail to demonstrate this property. It is the recognition of these exceptions which casts doubt on the correctness of Bull's interpretation of the mechanism explaining the phenomenon of removal of bacteria from the blood stream.

[88] Jour. Exper. Med., 1915, 22, pp. 475 and 487.
[89] Comp. rend. Soc. de biol., 1918, 81, p. 53; Presse méd., 1918, 26, p. 597.
[90] Jour. Exper. Med., 1917, 26, p. 441.
[91] Ibid., 1918, 27, p. 1.

In normal guinea-pigs the bacilli are loosely aggregated without being truly agglutinated and yet they promptly collect in the lung, liver, spleen, etc. In a general manner one gains the impression from properly stained smears prepared from the organs that a true agglutination has not taken place, but that physical disturbances in the colloidal suspensions, the blood and the bacteria, are responsible for the aggregations of bacilli. In some respects it is difficult to distinguish agglutinations in vivo from the phenomenon of the concentration of foreign particles in the viscera observed by Werigo,[92] in normal animals. In immunized rabbits and guinea-pigs the removal of bacteria takes place more rapidly, but we fail to note any differences in the formation of the clumps, which would definitely indicate true agglutination. As a rule, the aggregations are not more compact, nor do they consist of more bacteria. Our observations record the fact that the bacteria disappear from the circulation and that this disappearance is frequently followed in the normal animal by the discharge of the organisms into the hepatic duct bile. Whether this removal is the result of in vivo agglutination or the action of the blood platelets, or purely a dispersion phenomenon of the two colloids, has to be decided by further investigation.

The behavior of the leukocytes as illustrated in charts 1, 2 and 3, deserves some consideration, as it may assist in the explanation of the ultimate fate of the bacilli accumulated in the viscera. The observations made on animals which, on account of the hepatic duct fistula, exhibited a postoperative leukocytosis, confirm the well-known fact that the intravenous injection of bacteria or foreign protein causes a leukopenia in the peripheral circulation notwithstanding the postoperative leukocytosis. This condition is again followed in a few hours by a considerable leukocytosis. In the smears 10 to 15 minutes after the injection the majority of the white cells were mononuclear forms. The studies of Goldscheider and Jacobs,[93] Werigo,[92] Schwarz,[81] C. W. Wells,[94] Sellards and Baetjer,[95] Jolly,[96] Nagao [97] and others lead to the conclusion that the leukopenia is the result of the accumulation of the polymorphonuclear leukocytes in the internal circulation, especially in the liver, spleen and lungs. The view of an uneven dis-

[92] Ann. de l'Inst. Pasteur, 1892, 6, p. 478.
[93] Ztschr. f. klin. Med., 1894, 25, p. 373.
[94] Jour. Infect. Dis., 1917, 20, p. 219; 1918, 20, p. 502.
[95] Bull. Johns Hopkins Hosp., 1918, 29, p. 135.
[96] Compt. rend. Soc. de biol., 1918, 81, p. 756.
[97] Jour. Infect. Dis., 1920, 27, p. 327.

tribution of the leukocytes in experimental typhoid infections is, however, not shared by Studer,[98] by Tachigara and Miura,[99] by Pepper and Miller,[100] and by others, mainly on account of their inability to demonstrate at the low point of the leukopenia an accumulation of leukocytes in the internal organs. The leukopenia is, according to Studer, who injected his animals subcutaneously, the result of a functional disturbance provoked by the typhoid toxin on the bone-marrow and on the lymphatic tissues. A similar opinion has been expressed by Tachigara and Miura. In this connection we recall some observations made in the course of studies on hyperleukocytosis (Hurwitz and Meyer,[101]) following the injection of living typhoid bacilli. During the period of leukocytosis the increase in the percentage of young forms of leukocytes or metamyelocytes is so great as to suggest that during the leukopenia some of the leukocytes are destroyed or that the typhoid bacillus injures the leukopoietic tissue, namely, the bone-marrow. This peculiar blood picture is frequently more marked in animals which have been injected with living typhoid bacilli. There are several explanations of the purpose of the localization of leukocytes in the viscera. The theory of a negative or repellant chemotaxis according to which the introduction into the general circulation of a foreign protein repels the polymorphonuclear leukocytes, causing them to find refuge within the capillaries of the internal organs, is theoretically possible. There is some doubt in the opinion of numerous observers as to the actual occurrence of a negative chemotactic action on leukocytes. The evidence adduced from our findings supports the conception of a positive chemotaxis in the viscera as advanced by C. W. Wells, Nagao (footnote 97, p. 351), and others. The disappearance of the polymorphonuclear leukocytes from the blood stream was frequently preceded by the clumping of the bacteria and their removal to the liver, spleen, etc. This fact is best shown in table 3.

Smears from the lung and liver demonstrate the well-known phagocytosis described by Bull.[88] It is obvious that the leukocytes perform a definite function, at least during the first 2 hours after an intravenous infection, in removing and distributing to the detoxicating organ—the liver—a considerable proportion of the typhoid bacilli. The bacterial protein liberated during the course of phagocytosis and

[98] Thesis, University of Zürich, 1903.
[99] Mitt. a. d. med. Fakult. a. k. Univers. z. Tokyo, 1917, 17, p. 539.
[100] Jour. Infect. Dis., 1916, 19, p. 694.
[101] Jour. Exper. Med., 1916, 24, p. 515.

phagolysis may exert an additional chemotactic effect on the leukocytes or may in turn injure the bone-marrow, as suggested by Studer and others. The latter point can, however, not be decided from the data at our disposal, and the action does not occur during the first 2 hours.

This interpretation of the leukocyte counts affords no support for the conclusion that the leukopenia in typhoid fever is explained on the same basis. As far as the leukocyte counts of the peripheral blood is concerned, the available evidence indicates that the absolute diminution is much less than one has ordinarily supposed. Moreover, the spleen tumor is not the result of an accumulation of leukocytes as assumed by C. W. Wells, but due to a hyperplasia and activity of the reticulo-endothelial macrophages accompanied by a toxic inhibition of the leukopoietic function as stated by F. A. Evans,[102] S. Gräff [103] and others. Moreover, the recent observations of Askanazy [104] and of Gräff indicate that the typhoid bacillus and its toxin exert primarily specific positive chemotactic influence on the endothelial cells, while the leukocytes only become engaged later in the course of the disease when the necrotic tissues saturated with bacterial protein act as chemotactic foreign bodies. This local accumulation of leukocytes for the purpose of digestion of the necrobiotic foci has no appreciable influence on the blood picture of the peripheral blood.

TABLE 3

RABBIT 1265 INJECTED WITH 345,000,000 TYPHOID BACILLI. 1 C C OF THE SERUM OF THIS RABBIT DESTROYS IN 5 HOURS 1,000,000 TYPHOID BACILLI

Time After Injection	Heart Blood Contained	
	Bacteria per C c	White Cells
30 seconds	3,200,000	11,300
2 minutes	800,000	14,600
4 minutes	400,000	10,500
10 minutes	34,000	
11 minutes	34,800	4,200
15 minutes	6,500	2,600
20 minutes	800	1,400

Experiments on rabbits even when conducted with paratyphoid bacilli cannot be chosen for a reproduction of the condition in man. The removal of the bacteria and the subsequent leukopenia resulting from an intravenous injection is nonspecific and can be observed following the injection of any micro-organism. Even the progressively

[102] Bull. Johns Hopkins Hosp., 1916, 27, p. 356.
[103] Deutsch. Arch. f. klin. Med., 1918, 125, p. 352; and 126, p. 1.
[104] Askanazy, M.: Deutsch. med. Wchnschr., 1916, 42, p. 897.

invasive paratyphoid bacilli are actively phagocytized during the period of leukopenia, when the leukocytes and the clumped bacteria are brought in intimate contact. The ultimate fate of the gram-negative bacilli in the viscera may depend on some mechanism other than the ingestion by polymorphonuclears, namely, the endothelial cells or the bactericidal substances liberated by the leukocytes may play an important rôle in this problem.

CHOLECYSTITIS AS A RESULT OF GALLBLADDER WALL INFECTION

In the course of nearly 500 necropsies on rabbits infected by intravenous injection of typhoid bacilli, we noted in about 25% of the animals extensive inflammatory processes in the gallbladder wall. This organ was frequently adherent to the abdominal wall, the liver lobes, the stomach or even to the large intestines. Some rabbits killed from 8 to 10 days after the injection exhibited either a diphtheritic, necrotized or perforating gallbladder wall, the lesions being prominent particularly in the fundus region. Numerous small mural abscesses associated with hemorrhages were occasionally evident. In some experimental series the lesions named in the foregoing were more frequent than in others; in fact, in one series of 48 rabbits, the inoculation of agglutinated living typhoid bacilli produced in the gallbladder of 5% of the animals, blood-tinged biles with small or large blood clots. In a number of instances hemorrhagic ulcers or erosions were noted in the jejunum of the same animals. Histologically, the lesions originated from the terminal blood vessels of the mucosa. These and similar observations, the experimental results reported in paper IV, and the findings collected from rabbits infected intravenously with paratyphoid B. bacilli (Litch and Meyer [43]) left no doubt that the gallbladder wall is often seriously damaged in the course of a typhoid or paratyphoid infection. Careful culture studies supplied, moreover, data which indicated that gallbladder biles might be found sterile, while the walls yielded innumerable specific colonies. Furthermore, the findings of a large percentage of cholecystitis cases among immunized rabbits did not harmonize with our conception of a hemato-hepatogenous route of infection. It was obvious that the small number of typhoid bacilli which was eliminated in the bile could pass directly through the hepatic duct into the intestines and might never reach the gallbladder. These and other considerations

made it apparent that the conception of a hemato-hepatogenous route of biliary infection did not explain all of our results. An attempt was therefore made to verify the studies of Chiarolanza,[48] who demonstrated nests of bacilli lying in relation to the capillaries of the gallbladder wall, from which source the bile was apparently invaded.

The experiments of J. Koch and Chirolanza [48] have already been discussed and it has been concluded that for technical reasons they are open to serious criticisms, as are those dealing with the ligated cystic duct reported by R. Doerr.[1] A histologic study of the gallbladders removed at various periods after the infection has therefore been chosen as the most promising method of determining the possibility of infection of the bile by way of the gallbladder wall. Such an analysis gives not only definite information relative to the theory of wall infection, but also furnishes facts which help in the explanation of the exclusive persistence of typhoid bacilli in the gallbladder.

The literature contains reports of the histologic studies of Weinfurter,[58] Hailer and Ungermann,[105] Emmerich and Wagner,[106] Violle [15] for typhoid, and those of Creig [73] for cholera cholecystitis of the rabbit. As a rule, the chronic stage of the infection has been studied. The majority of writers mention cellular infiltration with collections of bacteria in the mucosa, and accept unreservedly the descriptions and interpretations of Chiarolanza. An excellent description of the gallbladder lesions found in rabbits infected with a specific cholecystotropic paratyphoid bacillus has been published by Fränkel and Much.[69] Bacterial thrombi have been observed by these investigators in the capillaries of the mucous membrane either at the top or at the base of the folds. Frequently wall abscesses and bacterial masses have been noted in the lymph vessels. These reports are in marked contrast to the repeated statements of Nichols [107] that he has been unable to find bacterial foci in the gallbladder wall.

In the course of our infection experiments we systematically collected and fixed the gallbladders, while warm, in Zenker's solution. Sections were stained with hematoxylin-eosine, or with dilute Manson's blue or carbolthionine. The gallbladders of 132 rabbits have been examined, but a few characteristic sections are chosen for a discussion of the most important findings.

[105] Arb. a. d. k. Gsndhtsamte, 1914, 47, pp. 303, 451 and 470.

[106] Centralbl. f. allg. Path. u. path. Anat., 1916, 27, p. 433.

[107] Jour. Am. Med. Assn., 1917, 68, p. 958; Nichols, H. J.; Simmons, I. S., and Stimmel, C. O.: Ibid., 1919, 73, p. 680.

The description of the microscopic structure of the normal gallbladder, as reported by Violle [15] and others, deserves one addition. In the majority of the gallbladders we found the villi of the mucosa located in the fundus portion to be long and irregular (fig. 1), resembling a papillomatous growth. The mucosa of the neck and the lateral walls, in contrast, have short and stumpy villi (fig. 2). The epithelium always exhibits a cuticular border, the cells are frequently of the goblet type, and in properly fixed material the lining is perfectly intact.

The pathologic changes, which we found in looking over many hundreds of sections, are definite and conclusive. In this connection it should be emphasized that the lesions to be described can only be found in 25% of the rabbits inoculated into an ear vein or by way of a radical of the portal vein. This statement applies particularly to the important lesions which develop between the 6th and the 72nd hour. The course of the wall infection can be discussed after studying the photomicrographs (in figs. 1 to 12), and is briefly as follows:

At the end of the 6th hour, after an intravenous injection of at least 5,000 million organisms in a rabbit weighing 2500 gm., the gallbladder bile may be sterile, but the wall may contain thousands of typhoid bacilli. A scrutiny of the villi of such a gallbladder may reveal dilated capillaries, perhaps nests of leukocytes and an edematous infiltration of the perivascular tissue (fig. 2). Typhoid bacilli can occasionally be demonstrated in the vascular endothelium, but quite regularly in the Kupffer cells of the liver. At the end of 24 hours a hemorrhage may occupy the tip of the villus and the adjacent epithelium is in a stage of necrobiosis. The cellular infiltration of the mucosa is confined to the necrotic villus (fig. 4). The bile contains culturally few bacteria, tinctorially a few clusters may be demonstrated. The focal necroses of the villi resemble the well-known lesions usually found in the liver of the same animals. The mucosa of the fundus is the place of predilection and the tissue destruction may be limited to one or two folds. It is self-evident that serial sections alone will reveal this type of wall infection. Toward the end of the 48th and 60th hour the focal necroses in the villi are in some animals quite numerous and may in certain cases be recognized macroscopically as small yellowish spots. The epithelium of the villi is frequently intact and shows normal nuclei. The photomicrographs (figs. 5 and 6) illustrate the location of the areas either in the top or the base of the villi. The center of the necroses consists of cellular débris, fibrin and

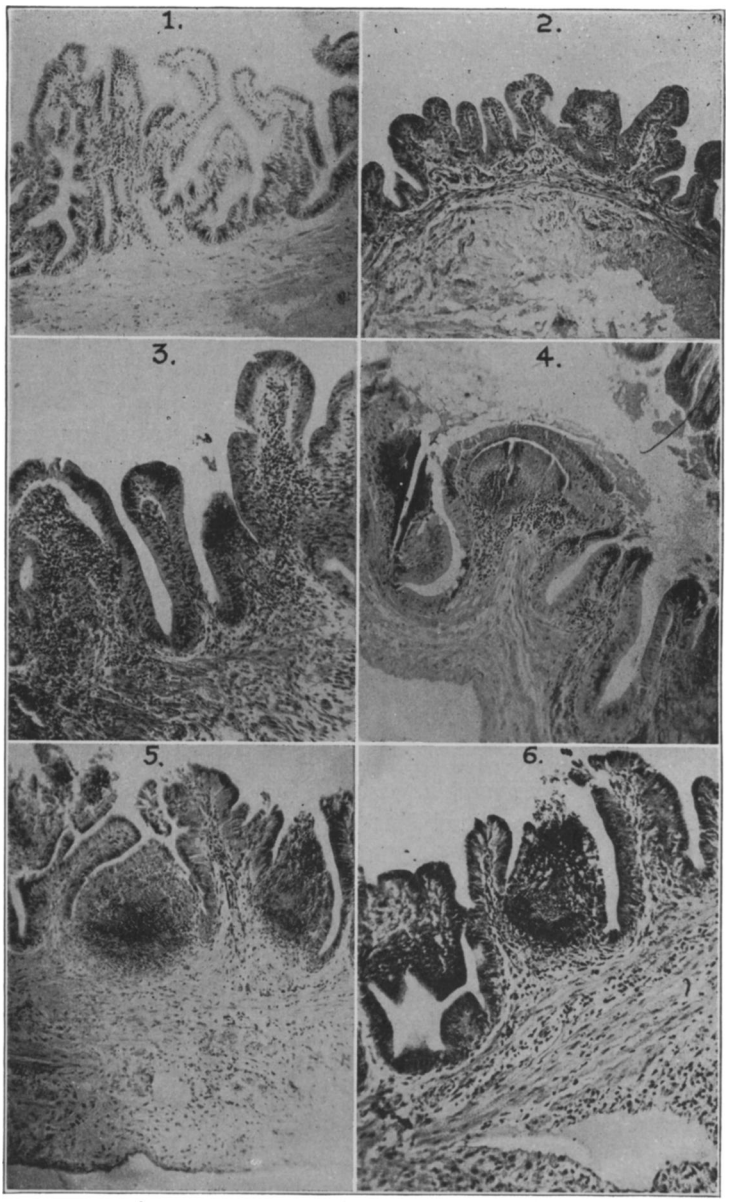

Fig. 1.—Rabbit 725; fundus of normal gallbladder.

Fig. 2.—Six hours. Rabbit 1701 A; intravenous 8 billion; green bile, sterile; wall innumerable colonies.

Fig. 3.—Twenty-four hours. Rabbit 1704. Direct gallbladder injection; 22 billion typhoid bacilli; colorless, limpid fluid with leukocytes, no blood.

Fig. 4.—Twenty-four hours. Rabbit 1707 (11). Six billion intravenous; bile deep green, viscid, 150 colonies per c c; wall ∞ colonies; macroscopically intact mucous membrane.

Fig. 5.—Rabbit 897; B. typhosus ½ blood-agar slant; bile deep green viscid, ∞ colonies from bile and gallbladder; subserous edema; mucosa slight erosions.

Fig. 6.—Rabbit 897; B. typhosus ½ blood-agar slant; bile deep green viscid, ∞ colonies from bile and gallbladder; subserous edema; mucosa slight erosions.

clusters of bacteria. Occasionally the demarcation and the discharge of the necrotic villi into the lumen of the gallbladder can be followed. Culturally at this stage of the infection the cystic bile may show one million bacteria per c c. The presence of a large number of bacilli in the bile leads to an edema and a diffuse cellular reaction of the entire mucosa of the gallbladder, while the localized necrosis may develop into a diphtheritic tissue defect, which extends into the muscularis, submucosa and serosa. In some instances a fibrinous pericholecystitis, even a perforation of the ulcerative, gangrenous inflammation has been observed. The formation of hemorrhages, the focal necroses and the diphtheritic lesions on the tips and at the base of the villi definitely suggest an embolic invasion of the terminal capillaries.

Theoretically it is remotely possible that the typhoid bacilli, which reach the bile by way of the hemato-hepatogenous route, multiply in the stagnant content of the gallbladder and invade the villi from within. Moreover, it is not unlikely that the bacteria can destroy the epithelial lining and enter the mucous coat in consequence of these defects. As the entire conception of the transverse infection rests on the conclusive demonstration of a primary focus in the capillaries of the wall, we have inoculated large doses (1 to 4 billion) of typhoid bacilli in 0.5 c c of saline directly into the gallbladder of normal rabbits in order to determine whether the gallbladder foci described in the foregoing could be developed in a similar manner by this route. The injections have been made with and without ligature of the needle puncture according to the method of Venema.[23] Six animals successfully operated on without hemorrhage into the gallbladder, have been killed 24 to 60 hours after the intracystic injection. Serial sections showed a complete absence of localized areas of necrosis in the villi. The histologic picture (fig. 3) is characteristic for a catarrhal cholecystitis. The epithelium is intact, invariably studded with emigrating leukocytes, and sometimes it is loosened from the mucosa. The latter is diffusely infiltrated with lymphocytes and polymorphonuclear leukocytes. The inflammatory process involves the lymphatic spaces and lymph vessels of the submucosa and serosa; in fact, the entire gallbladder wall is macroscopically edematous. The dilated lymph vessels and capillaries contain thrombi of leukocytes and lymph material. The lymphostasis resulting from the involvement of the lymphatic system extends along the bile duct into the portal tissues of the liver. Neither necrosis of the tips of the villi nor diphtheritic ulcerations analogous to illustrations 4, 5 and 6 can be detected. The typhoid bacilli and their products invade the wall

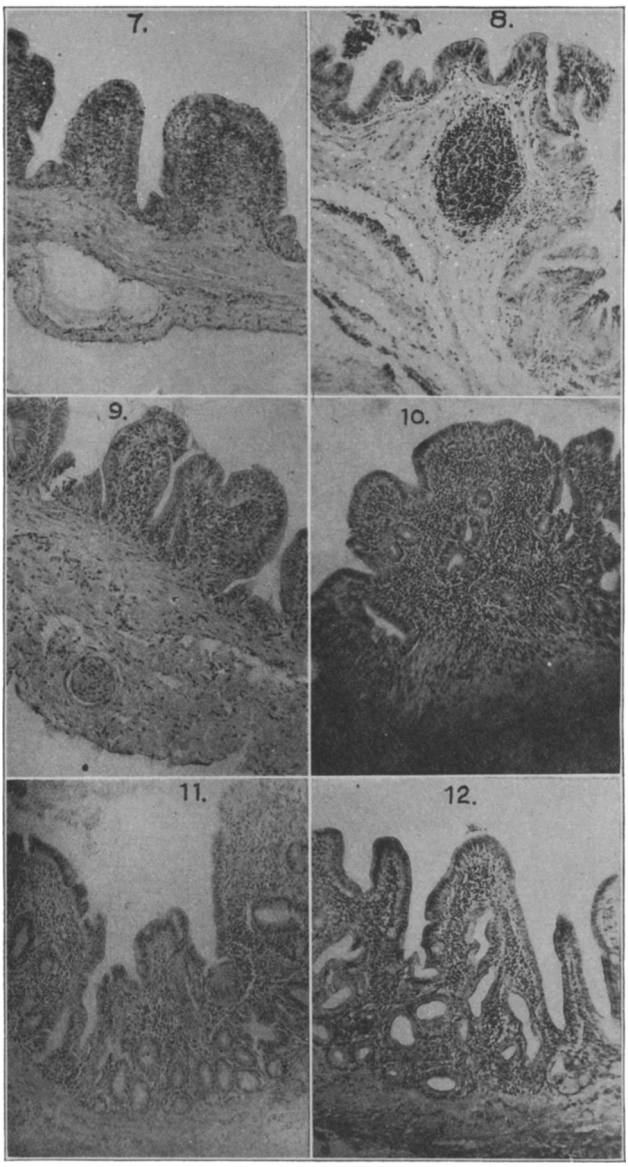

Fig. 7.—Rabbit 1021, 7 days, immunized, 10 billion agglutinated B. typhosus intravenously; gallbladder distended by colorless slimy pus, no erosions; extensive edema along extrahepatic ducts.

Fig. 8.—Rabbit 1095, 8 days, polyhomogenous B. typhosus, gallbladder distended, yellowish nodules and diphtheritic patches on mucosa; bile limpid, colorless. B. typhosus in all the organs.

Fig. 9.—Rabbit 1082, 31 days, 3 injections B. typhosus of recent isolation; loss in weight; intermittent shedding; irregular thickening of gallbladder wall, bile viscid, light green. B. typhosus in all the organs except heart blood.

Fig. 10.—Rabbit 1072, 32 days, polyhomogenous mixture of B. typhosus I. V.; intermittent shedding, cholangitis, empyema of distended thick walled gallbladder; B. typhosus in bile and wall, liver and duodenum.

Fig. 11.—Rabbit 705, 46 days; paratyphosus A; stool continuously negative; contracted gallbladder with 4 greenish soft stones size of a large pinhead; greenish watery bile. B. typhosus in bile and middle lobe of liver, duodenum.

Fig. 12.—Rabbit 15 A, 149 days, polyhomogenous I. V.; intermittent intestinal discharge of typhoid bacilli; gallbladder distended, 5 c c of light green bile with biliary sand about ⅓ of fluid. B. typhosus in bile and urine.

by way of the lymphatics; the resulting reactions involve these systems and not the blood vessels. Culturally, the bile invariably contains more typhoid bacilli than the gallbladder wall.

In studying this diffuse reaction it has been noted that the microscopic changes are the same whether the cystic bile has been infected by a direct injection or whether the bacilli enter the bile by way of the hemato-hepatogenous route and multiply in the bile. Moreover, the diffuse inflammatory reaction which follows the transverse embolic wall infection is of the same character. On the 3rd to 8th day after an intravenous injection of a large number of typhoid bacilli it is usually impossible to state from the study of a few sections whether the bacilli reach the gallbladder through the bile or through the blood vessels of the wall. In either case the bile in the viscus has become, on account of the addition of pus, lymph and blood fluids, an excellent substratum for the multiplication of the typhoid bacillus and invariably contains more germs per 1 c c than the wall. Their presence reacts on the layers of the wall in a manner absolutely identical with the one described for the intracystic injection. These facts explain in part the reports of Weinfurther,[58] Nichols [107] and others, who failed to observe the necroses, the diphtheritic inflammation of the villi, and the mechanism of the blood vessel injury which lead to the cholecystitis on account of their failure to make their histologic examinations sufficiently early.

The cellular reactions which develop in the course of the infection of the gallbladder wall are variable; they are usually diffuse, but may occasionally be localized in form of mural abscesses. The importance of these lesions on the persistence of the infection will be treated in a subsequent paragraph, but attention must be called at this point to a section of a gallbladder which was removed from a rabbit on the 30th day after infection (fig. 9). The connective tissue in the submucosa is increased, the blood vessel walls are either thickened and infiltrated with small lymphocytes or they exhibit organizing thrombi, all evidences of what is apparently a chronic inflammatory process. The origin of these extensive blood vessel lesions and their ultimate fate are difficult to determine in that we have not been afforded the opportunity of studying them at varying periods of their development. A systematic search will probably produce additional evidence to support the fact that the infection of the gallbladder wall plays an important rôle not only in streptococcic (Rosenow [67]), but also in typhoid and paratyphoid cholecystitis.

The typhoid bacillus reaches the gallbladder in human cases of typhoid regularly after the liver has been disabled by the poisonous products of the bacteria, and its presence there is evidenced by positive cultures or by a mild or moderately severe catarrhal cholecystitis (E. Fränkel,[108] Posselt[109]). According to the findings of numerous pathologists, the lesions present in the gallbladder and liver are certainly not as severe is frequently assumed by bacteriologists and epidemiologists. Purulent, necrotizing, inflammatory processes are infrequent and the formation of typhoid foci in the gallbladder wall supposedly of hematogenous origin are exceptionally rare. A general application of the conclusions of J. Koch[110] based on an exceedingly severe and unique form of typhoid cholecystitis is by no means justified.

From our own limited number of observations[111] and the evidence which we have been able to gather from the literature (Bindseil; Messerschmidt[112]; Goebel; Nichols, Simmons and Stimmel[113]; Küster, and Günzel[114]), it is evident that the embolic infection of the gallbladder wall plays an insignificant rôle in the human carrier state. A condition analogous to that found in man apparently exists in the guinea-pig. The microscopic and cultural study of a limited number of gallbladders derived from infected guinea-pigs convinces us that the wall is rarely, and then only slightly, infected through the bile. In fact, properly immunized guinea-pigs never develop gallbladder infections.

The factors responsible for the hematogenous infection of the gallbladder of the rabbit have not been studied with the desired thoroughness on account of the large number of animals required for such tests. It has been stated in connection with our work on paratyphoid B infections in these animals, that intravenous or intraportal injections are prerequisites for the development of a cholecystitis. Feeding or subcutaneous injection fails to cause an infection. Similar observations were made by Fränkel and Much[69] with a specifically elective para-

[108] Mit. a. d. Grenzgeb. d. Med. u. Chir., 1909, 20, p. 898.

[109] Ergebn. d. allg. Path. u. path. Anat., 1919, 19, pp. 351 and 471.

[110] Ztschr. f. Hyg. u. Infekionskrankh., 1908-09, 62, p. 1.

[111] We examined several gallbladders of convalescent typhoid patients which had been removed on account of gallstones. Histologically a diffuse infiltration of the mucosa and submucosa, or nests of round cells covered by an intact epithelium were observed. The cultural findings in one case also support the anatomic findings that the wall is not seriously involved, namely, the bile contained 30,000,000 typhoid bacilli per c c while 1 gm. of washed wall contained 240 bacilli.

[112] Ztschr. f. Hyg. u. Infektionskrankh., 1913, 75, p. 411.

[113] Jour. Am. Med. Assn., 1919, 73, p. 680.

[114] Ztschr. f. Hyg. u. Infektionskrankh., 1916, 81, p. 447.

typhoid bacillus. In experimental typhoid the individual susceptibility of the rabbits has interfered seriously in the numerous tests undertaken to determine some of the factors which lead to a wall infection. Typhoid strains which produce extensive liver necroses, provoke, as a rule, similar lesions in the gallbladder, provided the inoculated dose of bacteria is sufficiently large and made by intravenous, arterial or intrasplenic injections. Agglutinated bacteria or those grown on potatoes or blood agar give positive results more frequently than those cultivated in broth. Furthermore, starvation and changes in diet appear in some of the experimental series to be the factors conducive to wall infections.

THE FACTORS RESPONSIBLE FOR THE PERSISTENCE OF THE TYPHOID BACILLUS

Some writers on experimental cholecystitis conclude from their studies that the gallbladder is the only site in which the typhoid bacillus persists long after it has disappeared from all other parts of the body. These conclusions are based on a limited number of experimental animals and on incomplete tissue cultures. The recent reports of Hailer and Ungermann,[105] of Wagner and Emmerich,[115] fail to support this view. The first named writers found the spleen infected in 56%, the liver in 66%, and the kidneys in 46%; while the gallbladder or its contents harbored the typhoid bacillus in 93% of the instances. The data of Wagner and Emmerich demonstrate even more conclusively the participation of other organs as sites for the persistence of the bacilli in the rabbit. Animals killed on the 226th or 346th day harbored typhoid organisms in the spleen, lungs, liver and gallbladder. We collected at random the necropsy findings of 18 rabbits, which were killed or had succumbed to an intercurrent infection on the 64th to the 816th day after an intravenous inoculation. These animals were chosen because minutely detailed cultures were made on their tissues. The findings are shown in table 4. Additional data will be published in subsequent papers.

It is evident from table 4 that animals which succumb to intercurrent infections (due to B. cuniculisepticus, enteritis) or perhaps auto-infections, may exhibit an extensive dissemination of typhoid bacilli in the various viscera. Rabbits, on the other hand, which were in good health when killed, usually showed typhoid bacilli only in

[115] Med. Klin., 1916, 12, p. 819.

TABLE 4

ANATOMIC AND CULTURAL FINDINGS IN CHRONIC TYPHOID INFECTIONS OF RABBITS

Rabbit	When Killed After Infection	Anatomic Findings on Gallbladder	Bacteriologic Findings												
			Bile	Gall-bladder Wall	Liver	Spleen	Bone-marrow	Kidneys	Urine	Mesenteric Lymph Nodes	Lungs	Heart Blood	Duodenum	Jejunum	Ileum
840	180 days	Small, thick wall, bile light green with granular calculi.....	T	T	0	0	0	0	0	...	0	0	0	0	0
884	81 days	Distended, thick, light blue, granular sediment about ½ calculi	T	T	0	0	T	0	0	...	0	0	T	T	T
893	245 days	Distended, cystic duct blocked, light green with blackish calculi	T	T	T	0	T	0	0	T	0	0	0	0	0
10	99 days	Distended, thick wall injected, light green bile with coarse granular sediment	T	T	T	0	0	0	0	0	0	0	T	T	T
13	103 days	Distended, very thick wall, light green bile with coarse granlar sediment	T	T	0	0	0	0	0	0	0	0	0	0	0
19	103 days D. Pneumonia and pericarditis	Distended, very thick wall, purulent whitish with sediment...	T	T	T	T	T	T	—	T	—	T	—	T	T
800	83 days	Typical thick wall and light green bile, heavy sand-like débris	T	T	T	0	0	0	0	T	...	T	T	T	T
805	84 days	Distended, thick wall, light green bile with pus and sandy débris	T	T	0	0	0	0	0	...	T	...	0	T	TT
856	232 days	Thick wall, adhesions, light green bile with blackish calculi..	T	T	T	0	T	0	0	0	0	0	T	T	TT
865	409 days	Thick wall, light green, sand-like sediment with blackish calculi	T	T	0	0	0	0	0	0	0	0	T	T	TT
912	64 days D. pneumonia	Thick wall, purulent flocculent bile, yellowish concretions, extensive cholangitis	T	T	T	T	T	0	0	0	T	0	0	0	0
918	140 days	Thick wall, light greenish bile, sand-like débris.....	T	T	T	0	0	0	0	0	0	0	T	0	T
871	102 days D. pneumonia	Thick wall, adhesions, pure pus, and blackish débris, cystic duct obstructed	T	T	T	0	T	0	0	0	0	0	0	0	0
1034	249 days	Thick wall, yellow, slimy purulent bile.....	T	T	T	0	0	0	0	0	T	0	T	T	T
946	86 days	Thick wall, clear light green bile, cholangitic abscesses.....	T	T	T	0	0	0	0	0	0	0	T	T	T
1067	162 days	Thin walled, dark green.....	0	0	0	0	T	0	0	0	0	0	0	0	0
1036	251 days	Small, thick wall, few drops of light yellow-greenish sediment; viscus atrophic, cholangitis of the extra hepatic ducts.....	T	T	0	0	0	0	0	0	0	0	T	T	T
717 Para A.	816 days (relapse ?)	Size of a plum, thick wall, blackish granules in purulent light brown (largest gallbladder noted).....	T	T	T	T	T	0	0	T	T	T	T	T	T

the liver, bone-marrow and intestinal content. The persistence of the microbes is therefore not confined to the bile and gall-bladder wall. In one exceptional instance an animal came to necropsy in which the injected organisms were found only in the liver; and in another isolated animal killed on the 162nd day, only in the bone-marrow. Under special conditions, a prolonged localization in the kidneys can be demonstrated. In guinea-pigs a general dissemination and persistence in the tissues is the rule, while gallbladder lesions may be absent although typhoid bacilli are continuously discharged in the stools.

Particular study has been given to rabbits which show foci in the liver and bone-marrow. Thus far typhoid bacilli have not been found in the intestinal tract, bile or gallbladder of animals which carried the infection essentially in the remote viscera. Even liver foci have been recognized culturally, while typhoid bacilli have been absent in the cultures made from the bile and the intestines. In our experience the intestinal discharge of typhoid bacilli in the rabbit is, as a rule, associated with a gallbladder infection. It remains, therefore, to be proved, at least for this animal, that a closed liver focus or any distant focus, provided gallbladder infection is absent, is in a position to infect the intestinal tract through the bile, to which the organisms are supposed to have been carried by the blood or lymph stream. The factors responsible for the persistence of typhoid bacilli in the rabbit are in all probability confined to the gallbladder and to the extra- and intra-hepatic biliary system.

The question, "What is the average period of persistence of typhoid bacilli in the gallbladders of rabbits and guinea-pigs infected by way of the blood stream or by direct injection," must be answered before an analysis of the causes can be undertaken. Most observers who have kept rabbits for periods extending over 30 days agree that the localization of the bacteria is a temporary one in 60 to 70% of the animals. Lemierre and Abrami [47] have noted a restitution of the inflammatory process on the 6th day, while Lentz, Hailer and Wolf [60] have observed recoveries at the end of the second and third week. Morgan [50] found at the end of the 4th month 3 out of 7 animals, and Doerr 1 out of 2 rabbits, free from typhoid bacilli. According to Hailer and his associates,[116] the duration of the infection depended on the degree of the lesions; in the course of 2 to 3 weeks, 40% of the rabbits lost their infection. On direct inoculation into the gallbladder they failed in

[116] Arb. a. d. k. Gsndhtsamte, 1914-15, 48, p. 80.

several rabbits to recover the organisms on the 32nd and 38th days. Similar findings were made by Emmerich and Wagner.[117] Our observations on a series of 131 successfully infected rabbits showed that 85% gave positive cultures on the 26th to 34th day when the bile samples were obtained by gallbladder punctures; 10% of the animals exhibited thickened gallbladder walls, while 5% were macroscopically normal. Infected animals when kept under observation for 80 to 100 days recovered from the specific cholecystitis in 72% of the instances. After a lapse of from 120 to 450 days a series of 35 rabbits showed typical thickened gallbladder walls, but the typhoid bacillus was isolated only from 10 to 27% of the animals. The cultures of several gallbladders gave a growth of indifferent streptococci. Based on these data, it must be concluded that successful typhoidal gallbladder infection in rabbits is frequently a temporary process. Chronic infections comparable with those of the human carrier at the end of 3 months are found only in 10% of the infected rabbits. As a rule, direct intracystic inoculations are conductive to a prolonged persistence (for more than 100 days) in a higher percentage of cases than intravenous injections. This corresponds to the findings of Wagner and Emmerich. Rabbits with chronic infections are always in perfect health and gain in weight. They are occasionally the victims of intercurrent infections, but not to a greater extent than control animals.

It is obvious that these observations in rabbits are in many respects analogous to those in human carriers. It may be mere coincidence that the percentage of 11.6% of typhoid carriers found in India by Semple and Creig [118] corresponds rather closely with the data collected on rabbits. The majority of the rabbits can, following the classification of Sacquépée,[119] be placed in the group of "temporary" or "convalescent" carriers; while 10% develop into "chronic" carriers. It is a well-known fact that typhoid bacilli may persist in the stools of human beings for 3 months after apparent recovery from typhoid or paratyphoid fever, and according to Reibmayr [120] 25% of these convalescent carriers free themselves of the infection spontaneously. Moreover, Goubau [121] has shown that autovaccination clears such carriers of the bacteria much sooner than when they are left untreated.

[117] Centralbl. f. allg. Path. u. path. Anat., 1916, 27, p. 433; Med. Klin., 1916, 12, p. 74.

[118] Scientific memoir by officers of the Medical and Sanitary Department of the Government of India No. 32, Calcutta, 1908.

[119] Bull. Inst. Pasteur, 1910, 8, pp. 1 and 49.

[120] München. med. Wchnschr., 1918, 65, p. 670.

[121] Arch. Méd. Belges, Paris, 1917, 70, p. 590.

It must be borne in mind by every experimenter that spontaneous recovery of the temporary carrier state in rabbits is rather the rule than the exception. These animals are therefore unsuitable for practical chemotherapeutic tests. Chronic carriers, particularly rabbits, infected by direct inoculation of the gallbladder, develop lesions which cannot be treated pharmaceutically and usually present a therapeutic difficulty which is rarely encountered in human cases. Appreciating these limitations, it seems obvious that therapeutic results obtained in rabbits should not be unreservedly applied in drawing analyses as to the possibility of treating similar conditions existing in man. The proper procedure in studying the pharmacologic influence of various substances on typhoid carriers consists in testing them first on dogs with biliary fistulas, followed by experiments on chronic rabbit-carriers infected by the intravenous route. Then human cases, on whom a cholecystotomy has been done and drainage instituted, should be investigated in order that the bile secretion can be tested chemically as well as bacteriologically. Finally the same problems should be studied in chronic human carriers. In all typhoid cases an attempt should be made to prevent by proper treatment (high calory diet instead of starvation, which favors biliary stasis) the development of a carrier state. Carriers should be treated in the earliest possible stage by intensive autovaccination. For this purpose a few chemicals have been suggested, but their value has not been definitely proved. A prolonged carrier state usually leads to severe lesions which cannot be influenced by such procedures. The radical removal of the gallbladder is then not only from a hygienic standpoint, but also on account of the frequent presence of, or tendency toward, gallstones, in the interest of the carrier himself.

Having recognized in the rabbit the occurrence of temporary and chronic gallbladder infections, it is important to determine, if possible, the causes responsible for these variations. In this connection the various routes leading to gallbladder infections deserve further attention. A hemato-hepatogenous elimination of the bacteria is suspected of causing a temporary cholecystitis, while a wall invasion results in a chronic persistent infection. It has been repeatedly stated that the inoculation of several billion typhoid bacilli is necessary to produce an experimental gallbladder infection. In case this particular prerequisite is not fulfilled, neither a discharge of bacilli through the bile nor an embolic invasion of the wall takes place. It is known from our own observations and those published by others, that the bacterial

elimination in the bile is temporary and, as a rule, in the first 6 hours comparatively few typhoid bacilli reach the bile in the gallbladder, where unhindered multiplication can ensue. The few organisms which enter the bile are probably rapidly discharged into the intestines, as is evidenced by the findings in spontaneously and artificially produced (feeding) paratyphoid B infections (Litch and Meyer [43]). In case focal lesions, which communicate with the biliary passages, are established, as can be proved by intrahepatic injections (Hailer and Rimpau, personal observations), the elimination of large numbers of typhoid bacilli may be continuous for several days. Injury of the liver is accompanied by intoxication, with numerous physiologic and metabolic disturbances, one of which is biliary stasis. The bacilli reaching this viscus may remain, therefore, a sufficiently long period to permit of multiplication in a medium excellently suited for this purpose. In turn, the inflammatory reaction of the mucous membrane contributes an admixture of cells and lymph material, which favors the growth of the organisms in the cystic bile. Anatomically a catarrhal cholecystitis with little or no involvement of the submucosa and muscularis may be recognized. In these cases cultures of the bile give innumerable colonies of B. typhosus, while the washed wall is nearly sterile. This development of a mild gallbladder infection can be readily studied by direct inoculation of a small number of typhoid bacilli into the gallbladder. The degree of inflammation is moderate and the process involves mainly the subepithelial lymph system of the mucosa with a temporary lymphostasis in the adjacent systems of the muscularis and subserosa (fig. 7). Rabbits harboring such gallbladders are in the group of temporary carriers. At necropsy on the 10th to 20th day one finds a normal sized gallbladder with a slightly thickened wall which appears rather light in color. The bile is light or deep green, limpid, but contains no concretions of mucus. The P^{+}_{H} of the bile is less than 7.2 and changes rapidly to P^{+}_{H} 8.0-8.6. Cultures give typhoid colonies varying in number from 10 to several hundred per loop of bile. A careful histologic study of a large number of such gallbladders invariably shows an intact epithelium with lymphocytic infiltration of the mucosa, moderate proliferation of the connective tissue of the submucosa and a well preserved muscularis; no primary wall infection can be detected. The anatomic evidence supports the contention that these types of subacute cholecystitis may heal in from 2 to 4 weeks and then terminate the carrier state.

The etiology of the prolonged, temporary (50 to 100 days) or the chronic carrier state cannot be determined with certainty from our material. However, certain factors conducive to the persistence of the infection and the chronicity of the process can in a number of cases be definitely recognized. Diphtheritic foci of the gallbladder mucosa discharging clusters of typhoid bacilli into the bile are usually associated with liver lesions. The bile is being seeded from these numerous extensive foci. The inflammatory reaction resulting from this heavy and continuous infection is most severe as can be seen in gallbladder specimens removed on the 6th to 10th day of the disease. In exceptional instances the wall may show deep-seated abscesses (fig. 7). Usually the mucosa is partially necrotic, covered with leukocytes and fibrin; the submucosa and serosa are infiltrated with cells and fibrin and may even show small and large hemorrhages. The intrahepatic bile ducts not infrequently exhibit a marked inflammatory reaction. The bile is purulent, quite often blood tinged. These extensive changes may lead to adhesions or in exceptional cases to perforation. It is quite evident that such a profound involvement of the biliary system produced various anatomic and physiologic conditions, which favor the continuation of the infection. In chronic rabbit gallbladder carriers one of two findings is quite constant, namely, (1) empyema of the viscus with severe inflammation of the wall or (2) biliary sand or small calculi. The macroscopic findings of a number of gallbladders are reported in table 4. Histologic studies have been made on fresh tissue. Specimens with denuded mucosa are of questionable value and have not been used. The main findings are briefly as follows:

The outstanding features of the microscopic picture of the gallbladder showing an empyema is the thickening of the mucosa and submucosa to 4 to 8 times its normal dimension. The papillae of the mucosa are stumpy and diffusely infiltrated with lymphocytes. Occasionally nodular areas resembling lymph follicles can be observed. The covering epithelium exhibits a marked hyperplasia, but is intact. The cellular infiltration extends to the muscularis, which is either atrophic or its oblique fibers are increased in numbers (fig. 10). The stroma of the fibrous serous coat is enormously thickened and permeated with nests of lymphocytes and leukocytes. Frequently the wall of the blood vessels is thickened and infiltrated. The inflammatory process involves the cystic duct and frequently the extra- and intra-hepatic biliary system. A distinct cholangitis is found in 50% of the rabbits

killed between the 50th and 100th day of the infection. The bile is stringy, purulent and colorless. On standing, leukocytes and some biliary sand are sedimented (from 1/2 to 2/3 of the fluid bulk).

The gallbladder of the animals examined after the 30th day of the infection and that of every rabbit which harbored the typhoid bacilli for more than 100 days exhibit additional noteworthy microscopic changes. The thickness of the wall is not materially increased, but the mucosa exhibits elongated proliferations of the papillae. The entire mucosa coat contains numerous glands, which frequently show papillomatous extensions into the muscularis and submucosa resembling an adenomatous growth. Hypersecretion of mucus is distinctly visible, (figs. 11 and 12). An intact but hyperplastic epithelium covers a diffusely cellular-infiltrated stroma. The connective tissue growth in the serous and even the muscular coat is extensive; the lymph cell infiltration is comparatively slight. Round cells are, however, frequent in the hepatic connective tissue. The bile is rather viscid, the P^{+}_{H} is above 7.8, contains either a large amount of coarse, sand-like biliary concretion or even one or several well formed facetted stones. The latter may be black and friable. The findings are characteristic of those found in chronic cholecystitis. Invariably cultures prepared from the bile, stones, and wall give abundant colonies of B. typhosus. It has been impossible to demonstrate conclusively by tinctorial methods the bacilli in the tissues.

About 40% of our carriers killed before the 100th day exhibited the lesions of an empyema of the gallbladder, which explains readily the persistence of the bacteria in the biliary system. The empyema develops in consequence of an obstruction of the cystic duct or from a loss of contractibility of the wall. The latter may follow or precede the obstruction and is probably caused by the extensive proliferation of the connective tissue, submerging the important longitudinal and oblique muscle fibers. According to the available notes, cultures from the duodenum are frequently positive. Evidently the bacteria are eliminated with the hepatic duct bile from certain intrahepatic foci, because the inflammation in the cystic duct is sometimes so profound as to prevent a discharge of the gallbladder contents. These hepatic areas of necrosis or the microcholangitic abscesses are probably analogous to the lesions described by Blachstein,[7] and they explain the occasional persistence of typhoid bacilli in the stools of rabbits after cholecystectomy which does not always cure intestinal carriers (Loele [122]; in exper. 1 and 2).

Exper. 1.—Rabbit 1625, weighing 2,175 gm. was inoculated on Aug. 8 and 9, 1918, with 1 mg. of uranium nitrate, subcutaneously. On Aug. 20, 1918, it received an injection of 1,500 million B. typhosus, grown on potatoes, directly into the left kidney. The stool and urine were positive on Aug. 29, Sept. 2, 11, 15, 23 and Oct. 1. On Oct. 3 a complete cholecystectomy of an enlarged typical gallbladder was performed. The stool was negative on Oct. 4, 5, 6, 8, 9, 15, 25 and Nov. 10; while the urine was positive on Oct. 4, 5 and 8, and negative on Oct. 9, 15, 25, 30, and Nov. 10. At necropsy, Dec. 24, 1919, all the organs were found sterile.

Exper. 2.—Rabbit 1630, weighing 2,175 gm., was inoculated Oct. 8 and 9 with 1 mg. of uranium nitrate, subcutaneously. On Aug. 20, 1918, an injection of 1,500 million typhoid bacilli grown on potatoes was made directly into the left kidney. The urine was positive on Aug. 23, 29, Sept. 8, and Oct. 1. The stool contained bacilli on Aug. 29, Sept. 8, and Oct. 1. A complete cholecystectomy was performed on Oct. 3, 1918. The gallbladder was typical; the bile contained 3,200,000 bacteria per c c and the wall 3,500,000 per 100 mg. Recovery from the operation was rapid and complete. Urine cultures remained negative, while the stool cultures were positive on Oct. 4, 5, 6, 8, 15, 22, 28, 30, Nov. 10, and then remained negative on daily examination. At necropsy on Dec. 21, or 78 days after the operation, B. typhosus was isolated from the liver, the left kidney, the left bone marrow and a small abscess between the stomach and the liver. The intestinal tract was negative for B. typhosus.

From these experiments it is evident that the removal of the gallbladder does not always cure the carrier state, although the observations are not sufficient to warrant absolute deductions. In this respect the findings are analogous to those reported for human carriers, namely, cholecystectomy does not always cure intestinal carriers (Loele;[122] Fromme;[123] Schultze;[124] Nichols, Simmons and Stimmel [107]).

The bile of "chronic carriers," that is, rabbits which remained infected 100 to 816 days, contained invariably yellow-greenish biliary concretions, or blackish calculi. It appears of interest to consider briefly the nature and origin of these stones. The small concretions are microchemically composed of lime salts, traces of bile pigment and organic material, but no cholesterol. They are "Entzündungsteine" in the sense of Aschoff and Bacmeister,[125] or calcium concretions, which develop as a result of the inflammatory process. It is well known and has been repeatedly shown that a chronic cholecystitis leads to an hyperplasia of the mucous glands and to an excessive production of biliary mucus. Aschoff believes this mucus to be very rich in lime salts. Lichtwitz and Bock [126] consider the calcium content of the bile

[122] Deutsch. med. Wchnschr., 1909, 35, p. 1429.
[123] Deutsch. Ztschr. f. Chir. 1910, 107, p. 578.
[124] Centralbl. f. d. Grenzgeb. d. Med. u. Chir., 1913, 49, p. 1892.
[125] Die Cholelithiasis, Jena, 1909.
[126] Deutsch. med. Wchnschr., 1915, 41, p. 1215.

to be normal. Its precipitation, however, is facilitated by the excessive and concentrated glandular secretion.

Irrespective of the uncertainty relative to the origin and the physical-chemical factors which lead to the precipitation of the calcium salts of the bile, it has been definitely proved (Rosenbloom [127]) that biliary calculi composed of lime are regularly due to an infectious process. The conditions favorable for the development of such stones are unquestionably present in the chronic cholecystitis of the rabbit. Cal-

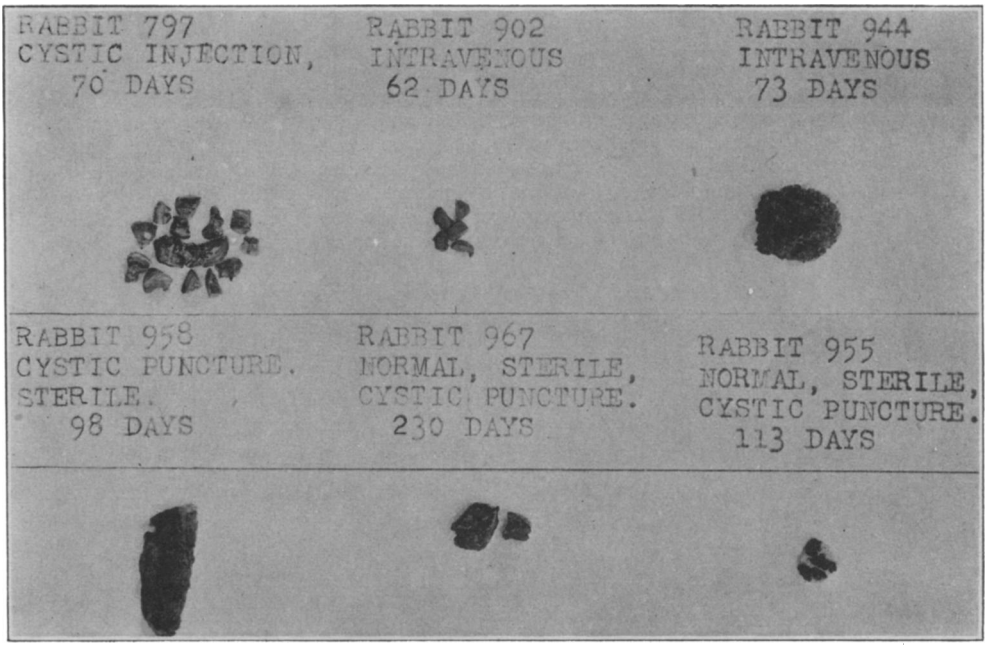

Fig. 13.—Stones (actual size) originating in gallbladders of rabbits inoculated intravenously with typhoid bacilli.

culi once formed favor the perpetuation of the inflammatory process and with it the persistence of the typhoid bacillus. The precipitated calcium salts are in part also responsible in carrier rabbits for the well-known low H-ion concentration of the cystic bile exposed to air for a few minutes. The large black or greenish stones have, in our opinion, a different origin. They are soft and friable; chemically they are composed of protein material (30 to 60%), some bile pigment and at times

[127] Jour. Am. Med. Assn., 1917, 69, p. 1765.

traces of lime salts. Certain stones (example shown, natural size, in fig. 13) originate in the gallbladders of rabbits which have been inoculated intravenously with typhoid bacilli. They are likewise found in this viscus after a direct inoculation by puncture of the gallbladder. It has been noted that blood clots develop quite frequently subsequent to a needle puncture or the application of a ligature. Hemorrhages and blood extravasations also result, in intravenously inoculated rabbits, as the consequence of the diphtheritic, necrotizing cholecystitis. The albuminous, organic nucleus of these stones is therefore primarily one of these blood coagulums, which in some cases may secondarily become impregnated with calcium salts (fig. 13, rabbit 944). It is obvious, that absorption of this type of stone is practically impossible and their presence assists in the prolongation of the cholecystitis and the persistence of the infection. This interpretation probably applies to the frequent findings of biliary calculi of this type in the gallbladders of rabbits infected by direct cystic injections (Emmerich and Wagner).

The concretions ordinarily found in rabbits should not be compared with the cholesterol stones of man. This fact has been emphasized by Klinkert,[18] who repeated the experiments of Cushing,[128] in which the gallbladder was first injured by compression with forceps, and the animal subsequently infected by an intravenous injection of typhoid bacilli. Bilirubin-calcium calculi could be found on the 54th day after the operation. Pure cholesterol stones artificially placed in the gallbladders of rabbits are, in our experience, gradually dissolved. By feeding cholesterol or lanolin we succeeded in producing pure cholesterol calculi, as will be discussed in paper VI of this series. Biliary calculi have been observed occasionally in rabbits infected with typhoid or colon bacilli and cholera vibrios (Richardson[1] Cushing;[128] Doerr;[1] Forster and Kayser;[46] Creig[84]). Spontaneous stone formation without previous bacterial injury of the gallbladder wall has not been reported. The absence of calculi in the rabbits killed before the 30th day (our own observations are supported by those of Hailer and Rimpau) and their constant presence in animals necropsied after the 100th day are significant. It justifies the conclusions that the chronic inflammation provoked by the typhoid bacillus is the cause of the precipitation of the lime salts. The resulting calculi in turn are probably the main factors favoring the persistence of the bacteria in the gallbladder.

[128] Bull. Johns Hopkins Hosp., 1899, 10, p. 166.

These findings indicate that the persistence of the typhoid bacilli in the rabbit's gallbladder is quite analogous to the carrier state in man. This latter condition is, according to the observations of Nichols, Simmons, and Stimmel,[113] of Messerschmidt,[112] of O. Mayer,[129] and others, associated in 65 to 90% of the cases with gallstones, while the remaining 10 to 35% of human carriers invariably show a distinct chronic cholecystitis or an empyema of the gallbladder. The excellent summaries of Posselt[109] and of Wagner and Emmerich[15] supply additional information for this comparison and should be consulted by those interested in the subject of cholecystitis caused by typhoid fever.

In the preceding discussion the function of the bile has not been considered as a factor controlling either the development or the persistence of a carrier state in rabbits. Comparatively recently Nichols expressed the opinion based on test-tube experiments that the biles of certain rabbits possess an inhibitory or even an antiseptic action. Our detailed studies reported in paper VI, VII and VIII, fail to support this contention. In fact, it is proved that the cystic bile of rabbits is particularly suited for the growth of B. typhosus.

TABLE 5

AGGLUTINATION OF TYPHOID BACILLUS IN CARRIERS AND RECOVERED CARRIERS

Carrier		Recovered Carriers	
99 days	1:1.000	134 days	1:2 000
103 days	1:1 000	149 days	1:2,000
140 days	1:2,000	180 days	1:2,000
180 days	1:2,000	205 days	1:600
232 days	1:600	252 days	1:100
245 days	1:4,000	314 days	1:600
409 days	1:400	430 days	1:200

A comparative analysis of a limited number of serologic tests fails to suggest any relation between demonstrable immune bodies and the carrier state. This should be expected in the light of the data discussed in paper IV. The serum of chronic carrier animals agglutinates the typhoid bacillus in dilutions above 1:100 quite frequently in dilutions as high as 1:4,000. In comparison with the original titer determined 15 to 20 days after the infection, the agglutinating power of the serum is considerably diminished. After a lapse of 100 to 200 days the immune bodies remain, however, fairly constant. Recovered carriers differ in no respect from the animals harboring a focus of infection, as is illustrated by the figures in table 6. Complement fixing antibodies

[129] München. med. Wchnschr., 1914, 61, p. 1116.

disappear more rapidly than the agglutinins, but no differences are noted between the infected and the recovered animals. It is well known that in rabbits the disappearance of agglutinins following an infection is fairly rapid in the first 2 to 3 months, and may remain constant for over one year. Guinea-pigs behave differently; such animals harboring typhoid bacilli in their tissues may give negative Widal reactions on the 50th or 60th day after infection. It is evident that serum tests can neither be used to detect a carrier state in rabbits or guinea-pigs, nor can they explain the recovery or the persistence of the bacilli in the tissues and particularly the gallbladder. Experiments conducted several years ago have proved that cutaneous hypersensitiveness tests are more marked in infected than immunized rabbits. Skin tests may therefore be employed to advantage in the detection of the rabbit carrier state.

In conclusion, it can be stated that the temporary typhoid carrier state in the rabbit is probably the result of a hemato-hepatogenous infection of the bile. The resulting inflammation of the gallbladder is comparatively mild and a restitutio ad integrum can take place readily. The chronic carrier state is favored by a severe wall infection resulting from an embolic invasion of the terminal capillaries of the mucosa. The persistence of the typhoid bacillus is facilitated by an obstruction of the cystic duct, the development of an empyema and the formation of biliary calculi. These chronic infections can be produced more readily in rabbits resistant to the typhoid toxin or by the injection of an atoxic strain in doses of not less than 2 billion per kilogram.

GALLBLADDER INFECTIONS PRODUCED BY THE INOCULATION OF TYPHOID BACILLI INTO THE SPLEEN AND INTO THE GALLBLADDER CONTAINING GALLSTONES

Hailer and Ungermann [105] attempted by direct inoculation of B. typhosus into the liver, kidneys, duodenum, appendix and gallbladder to produce typhoid foci in which the bacilli were protected from the destructive influence of the blood serum (?). It was also expected that these areas would constantly reinfect the other tissues of the body and favor a more chronic infection than could be obtained by intravenous injections. With the exception of the direct inoculations into the gallbladder and perhaps the liver and kidney, neither of the procedures offered any advantage over the usual intravenous method. The injections into the intestinal tract gave inconstant results, while the liver injections provoked extensive necroses which had nothing in common

with the lesions found in man. Our experience with these methods was similar.

The explanation of the carrier state by Webb-Johnson [8] already referred to, suggested the advantage of direct inoculation of typhoid bacilli into the spleen. This is, however, not the only reason why this method has been tried. There are many arguments which may be advanced against the intravenous injections. In the first place, there is necessarily considerable dilution of the infective material and an extremely good opportunity for the operation of any deleterious action which the plasma and cells may possess. Moreover, it seems logical to introduce the infective agent into the locations which are involved in the spontaneous infections as exemplified in the experimental production of tuberculosis and syphilis by inoculation into the testes. The spleen is readily exposed by laparotomy under ether anesthesia, and subcapsular injections of 0.5 to 1.0 c c can be made without injury to the organ.

Thus far we have practiced this method on 16 large rabbits (average weight 2,240 gm.). They have all recovered from the operation; when killed 11 to 31 days after the injection, 8 animals harbored typhoid bacilli in the gallbladder, spleen, liver, bone-marrow and intestines. The intoxication has been slight in comparison to that of the intravenously injected animals. In this connection it should be emphasized that the number of typhoid bacilli introduced did not exceed 500 million. It is well known that this dosage of bacilli produces gallbladder lesions only in exceptional instances in small rabbits. The remaining 8 rabbits are still under observation and are apparently infected, judging from the presence of the typhoid bacilli in the stools. The local lesions produced by the infection are slight; a few adhesions of the splenic capsule to the peritoneal wall, a minor spleen tumor and a small area of necrosis are the only changes recorded on the 31st day. From an experimental standpoint this method is an excellent one and a high percentage of carriers with a low initial mortality can be produced. These results naturally incline one to accept the view of Webb-Johnson. The spleen of the human carrier may be the focus which continuously supplies the bile with the specific organisms. Typhoid bacilli have been found in the spleen of the majority of chronic human carriers, examined after death. Their presence has been attributed to auto-infection, but some of the patients unquestionably died from pneumonia, apoplexy (Günther and

Böttcher,[130] Kamm[131]), without symptoms of auto-infection. In rabbits, however, a chronic gallbladder infection has never been found to be associated with a splenic focus. In our experience the only positive spleen cultures obtained were made from animals which died from intercurrent diseases (table 4, 19, 64, and 816). It is, however, possible that the direct splenic inoculation, which reproduces the conditions in man more accurately than the intravenous method, may furnish conclusive support to the above theory. Such information can, however, only be procured from animals which have been kept under observation for several years. A final opinion concerning this interesting conception of the carrier state by Webb-Johnson will be rendered when the studies of the remaining 8 rabbits are completed.

There had been considerable discussion as to whether the typhoid bacillus is capable of exciting the formation of gallstones or whether the stones are usually preformed, and as such predisposed to a localization of the invading organism. Having satisfactorily demonstrated the lithogenic properties of the typhoid bacillus in the gallbladder of rabbits, it is of interest to test experimentally the second view. The experiments conducted for this purpose are not entirely satisfactory. The introduction of a stone invariably leads to a secondary invasion of the gallbladder wall or the bile by "indifferent" intestinal streptococci. Under these circumstances the viscus is predisposed to a subsequent infection and typhoid bacilli as well as streptococci thrive in the tissues. In some experiments the typhoid bacillus is isolated from the gallbladder wall, while the bile itself contains streptococci. Moreover, it is proved that a relatively small dose of bacteria may localize in the gallbladder in which a calculus has been previously placed. Two experiments of a series of 8 are presented below to illustrate the above statements.

Exper. 1.—Rabbit 1399, weighing 3,095 gm., was laparotomized on March 3, 1919, and a fragment of a sterilized cholesterol stone removed from a human typhoid carrier was placed in the gallbladder. On June 9, 1919, or 97 days after the operation, the animal was killed. The gallbladder was distended and filled with pus-like yellowish fluid containing small fragments of the inserted stone and fine sand-like débris. The wall was thick and fibrous, the mucous coat was injected and partially denuded. The serous coat was edematous and the periportal lymph nodes were soft and edematous. The other organs were normal. Streptococcus salivarius was isolated from the bile, gallbladder wall, regional lymph node and duodenum.

[130] Ztschr. f. Hyg. u. Infektionskrankh., 1911, 68, p. 439.
[131] München. med. Wchnschr., 1909, 56, p. 1011.

Exper. 2.—Rabbit 1323, weighing 3,675 gm.; on Nov. 8, 1918, a sterile stone the size of grain was placed in the gallbladder. On Dec. 27, 1918, the animal weighed 3,750 gm., and was injected intravenously with 1/100 slant or 400,000,000 typhoid bacilli. On Jan. 20, 1919, the rabbit had diarrhea and appeared sick. It was chloroformed. At necropsy extensive adhesions between the liver, stomach and omentum were found. The gallbladder wall was thick, and contained about 3 c c of turbid, viscid, brownish-yellow bile. The stone was partially disintegrated and pigmented. The mucosa was smooth; no erosions were visible. Spleen and lymph nodes were enlarged, and soft; a moderate catarrhal enteritis was present. The bile contained streptococci, while the gallbladder wall, the liver, right kidney, spleen and lung gave pure cultures of B. typhosus. The streptococcus grew in opaque, grayish-white staphylococcus-like colonies.

From these observations it becomes evident that streptococci regularly invade a gallbladder injured by an operative manipulation or when irritated by a calculus. The origin of these bacteria is probably intestinal and the route is a lymphogenous one. A subsequent typhoid bacillus invasion is unable to dislodge these streptococci, but fosters a prolonged sojourn and wide dissemination of B. typhosus in the body. According to Rosenow,[67] Brown,[132] Starr and Graham,[133] and others, streptococci are frequently found in acute and subacute cholecyctitis of man. The foregoing data suggest a method to reproduce these infections experimentally. In our experience streptococci may rep'ace a colon or typhoid infection in the gallbladder of a rabbit; it is therefore recommended that extreme caution be exercised in the interpretation of the presence of streptococci as an apparent selective affinity for the gallbladder. In order that this type of experiment may be of some clinical value it is advisable to extend the period of observation over several years. It is not unlikely that such studies may assist materially in an understanding of gallbladder disease of man.

SUMMARY AND CONCLUSIONS

The elimination of typhoid bacilli in the hepatic duct bile of normal and immunized animals has been studied on rabbits, guinea-pigs and dogs provided with temporary common duct fistulas. These experiments have demonstrated that more bacteria appear in the bile of normal rabbits inoculated intravenously with 8,000 to 24,000 million typhoid bacilli than in that of immunized animals of the same litter, provided the last inoculation of the vaccine is administered 20 to 30 days previous to the infection.

[132] Arch. Int. Med., 1919, 23, p. 185.
[133] Ann. Surg., 1918, 68, p. 188.

The elimination of the bacteria by the hemato-hepatogenous or descending route is immediate. The maximum number of colonies develop on the plates prepared with bile specimens collected between the first 5 to 15 minutes following the injection. In subsequent periods the number decreases rapidly and the discharge of bacteria may cease completely at the end of one hour. The ability of the individual rabbit to eliminate bacilli in the bile varies considerably. Even repeated inoculations of large doses (10 to 15 billion) may in a small number of rabbits never lead to a discharge of typhoid bacilli in the hepatic duct bile.

The transit of the bacilli from the hepatic blood vessels to the biliary capillaries is probably governed by the phagocytic action of the endothelial cells. Immunization of the rabbits prevents, to a certain degree, the passage of bacilli. In the immunized guinea-pig the endothelial barrier is exceedingly efficient and an intravenous injection of less than 100 million typhoid bacilli usually gives sterile bile cultures, while in normal guinea-pigs several hundred organisms are eliminated in the fistular bile following similar injections.

Rabbits which have been inoculated with dead or living typhoid bacilli and which have been injectetd intravenously with living typhoid bacilli on the 6th to 10th day after the last immunizing injection, discharge more bacilli than the normal control animals of the same litter. This exceptional behavior of the immunized rabbit is probably due to an incomplete restitutio ad integrum of the injured vascular endothelium. Complete recovery from bacterial vaccination, which evidently does not take place before the 10th day, as a rule prevents the transit of bacilli from the blood to the bile.

The excretion of typhoid bacilli in the hepatic duct bile of dogs is irregular and not definitely influenced by immunization. Injections of less than 10 billion bacilli in dogs varying in weight from 14 to 26 pounds fail to cause an elimination of bacteria.

The removal of bacteria from the circulation in the first 10 to 60 minutes may be the result of an in vivo agglutination, the action of the blood platelets, or purely a dispersion phenomenon of two colloids. Virulent paratyphoid B bacilli of rabbit origin are clumped as readily and disappear as rapidly from the blood as the noninvasive typhoid bacilli. The removal of bacteria from the peripheral circulation is decidedly more rapid in immunized than in normal rabbits and guinea-pigs.

The leukopenia of the peripheral blood following the injection of bacilli is the result of an uneven distribution of the leukocytes which are chemotactically attracted by the bacillary masses collecting in the viscera.

Histologic evidence is presented which indicates that the gallbladders of about one third of the rabbits injected with large doses of typhoid bacilli receive the infection through the terminal capillaries of the mucosa. Preparations made from gallbladders removed on the 24th to 72nd hour after the injection show necroses and diphtheritic inflammatory areas in the villi of the mucosa. The transverse route of bile infection through the wall occurs also after ligation of the cystic duct.

Direct gallbladder injections produce an infection of the wall along the lymphatic system of the mucosa, submucosa and subserosa. The epithelium remains intact and focal necroses of the villi are not observed. In case the typhoid bacilli reach the gallbladder by the hemato-hepatogenous route only, they multiply in the cystic bile, which is suitable for their development. The histologic changes produced in the wall are identical with those provoked by direct inoculation.

Serial sections of the fundus portion of the gallbladder wall removed on the 3rd to 5th day following the infection may indicate on histologic examination the route responsible for the gallbladder lesion, whether descending or transverse.

Rabbit typhoid carriers may be classified into temporary or convalescent and chronic carriers. Thirty to 40% of the intravenously inoculated animals recover from their infection in the first month after the injection. About 10 to 15% may retain typical bacilli in the gallbladder for 6 months to one year, occasionally even longer. The persistence of the micro-organisms depends on the degree of inflammation provoked in the gallbladder. In case the bacillary invasion of the bile is innocuous to the tissues or produces only slight catarrhal cholecystitis, a temporary infection will be noted. It is not unlikely that such a state is regularly the result in descending hemato-hepatogenous infections.

Chronic carriers result probably from embolic, capillary invasion of the wall, with subsequent transverse infection of the bile. Persistence of the bacteria is favored by the formation of biliary calculi (60 to 80% of the cases), by the extension of the inflammatory process to the cystic ducts, and by a severe cholecystitis leading to a loss of contractibility of the wall, followed by a state of empyema. The biliary

calculi consist usually of bilirubin-calcium material. The nucleus of this may be an unabsorbed blood clot. Cholesterol stones have not been found. Chronic gallbladder carriers frequently harbor typhoid bacilli in the liver, bone-marrow, lungs and intestines. In exceptional instances isolated foci of infection may be found in the bone-marrow and kidneys. The elimination of typhoid bacilli in the feces of rabbits is, however, practically always associated with gallbladder or bile passage infections. It remains, therefore, to be proved that a close liver focus or any distant focus, provided the gallbladder infection is absent, is in a position to infect the intestinal tract through the bile, to which the organisms are supposed to have been carried by the blood or lymph stream. This contention is sound, notwithstanding the fact that in a few instances it has been demonstrated experimentally that in certain rabbits with infected gallbladders and extrahepatic biliary passages, cholecystectomy does not prevent the elimination of typhoid bacilli in the stools.

Intrasplenic injections of typhoid bacilli produce regularly persistent gallbladder infection. The conception of the human carrier state advanced by Webb-Johnson is discussed.

The insertion of sterile gallstones into the gallbladder of rabbits leads to a secondary infection of this viscus by indifferent streptococci. Such gallbladders are predisposed to a localization of the typhoid bacilli introduced by way of the blood streams.

The typhoid bacillus reaches the gallbladder in human cases of typhoid fever regularly after the liver has been disabled by the poisonous product of the bacteria, and its presence therein is evidenced by positive cultures or by a mild or moderately severe catarrhal cholecystitis. According to the findings of numerous pathologists, the lesions present in the liver and gallbladder are certainly not as severe as is frequently assumed by bacteriologists and epidemiologists. Cholecystitis is a far more frequent complication during typhoid fever than is generally supposed, but purulent necrotizing inflammatory processes are not common, and the formation of bacillary foci in the gallbladder wall supposedly of hematogenous origin is exceptionally rare. Application of the conclusion of J. Koch based on an exceedingly severe and unique form of typhoid cholecystitis is by no means justified.